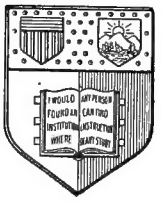
METHODS IN MICROSCOPICAL RESEARCH.

FLATTERS.



Methods

IN

Microscopical Research

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Microscopical Research

Vegetable Histology

BY

ABRAHAM FLATTERS, F.R.M.S.,

Lecturer and Demonstrator in Microscopy at the Municipal School of Technology, Manchester.

SHERRATT AND HUGHES
65 LONG ACRE LONDON W.
27 ST. ANN STREET MANCHESTER
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Preface.

THIS work is the outcome of the Microscopical Research Class conducted by me during the first session held in the new Municipal School of Technology, Sackville Street, Manchester, 1902-3. The course of instruction consisted chiefly in working out the internal structure of the higher vegetable types. The notes used were those I had written down from time to time in my private laboratory work. The photomicrographs are all from specimens collected, fixed, cut, stained, mounted, and photographed by myself, the reproductions from which are hand-painted, direct from the microscopical productions, by my artist assistants, under my supervision.

At the end of the session I was asked by several of my students to have the notes, formulæ, and methods of manipulation printed and, if possible, illustrated. To do this meant an enormous amount of additional work, especially in the production of suitable photomicrographs for publication, most of the sections for which had to be specially cut and stained to meet the requirements for reproduction in colours.

In the publication of this work I believe a long-felt want will be supplied, as I have endeavoured to lay down a system of work, which, if followed out by the student, will enable him successfully to carry the work through, from the collecting of the specimen to the finished microscopical preparation. Had such a work been at my service twenty years ago, I feel sure that I should have been spared years of persistent hard work and many disappointments.

The chief idea in the production of this work is to simplify the methods of microscopical research, in so far as relates to the specimens dealt with. There is not a single difficult problem in the work, if the student will make himself

acquainted with the art of cutting and staining sections. I trust the work will prove a help to the artisan and labourer students in microscopy, of which there are not a few, and to such I would say, be not discouraged, the author of this work issued from your ranks.

To Prof. F. E. Weiss, D.Sc., F.L.S., my thanks are due for his assistance and suggestions relating to the structural parts of the photomicrographs.

ABRAHAM FLATTERS.

20, Church Road, Longsight,
September, 1905.

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NINETY-TWO per cent. alcohol is the "ordinary" methylated spirits of commerce, free from the mineral additions as supplied by chemists. The strength of alcohol, as sent out from the distillery, varies from 88 per cent. to 92 per cent. In making up percentages, therefore, it is safest to calculate on a 90 per cent. strength, unless the student can obtain its specific gravity by means of the hydrometer. Percentages for all ordinary purposes may be obtained by volume as shown in the following table.

Table for Diluting Alcohol.

Desired strength of alcohol	100 volumes of alcohol of per cent.								
	90	85	80	75	70	65	60	35	50
	Require addition of volumes of water.								
85	6·8	—	—	—	—	—	—	—	—
80	13·8	6·8	—	—	—	—	—	—	—
75	21·9	14·5	7·2	—	—	—	—	—	—
70	31·1	23·1	15·4	7·6	—	—	—	—	—
65	41·5	33·0	24·7	16·4	8·2	—	—	—	—
60	53·7	44·5	35·4	26·5	17·6	8·8	—	—	—
55	67·9	57·9	48 1	38·3	28·6	19·0	9·5	—	—
50	84·7	73·9	63·0	52·4	41·7	31·3	20·5	10·4	—
45	105·3	93·3	81·4	69·5	57·8	46·1	34·5	22·9	11·4
40	130·8	117·3	104·0	90·8	77·6	64·5	51·4	38·5	25·6
35	163·3	148·0	132·9	117·8	102·8	87·9	73·1	58·3	43·6
30	206·2	188·6	171·1	153·6	136·4	118·9	101·7	84·5	67·5
25	266·1	245·2	224·3	203·5	182·8	162·2	141·7	121·2	100·7
20	355·8	329·8	304·0	278·3	252·0	227·0	201·4	176·0	150·6
15	505·3	471·0	436·9	402·8	268·8	334·9	301·1	267·3	233·5
10	804·5	753·7	702·9	652·2	601·6	551·1	500·6	450·2	399·9

To obtain any required quantity of any given percentage of alcohol from the above table, take for example, required 250 cc. of 60 per cent. of alcohol, how much 90 per cent. alcohol will be required. $1500 : 250 :: 100 = 166\frac{2}{3}$ cc.

Commercial methylated spirits may be used for scientific purposes when it is not necessary to use it for making up lower percentages by the addition to it of water, on the addition of which it becomes turbid, and specimens or sections when placed in it cannot be observed. For permission to use "ordinary" methylated spirits, free from the mineral additions, a permit must be obtained from the Excise authorities, for which purpose information may be obtained from any of the Excise offices.

The following table of factors for converting from one scale to the other will be found useful by the Student in working out formula, etc. :—

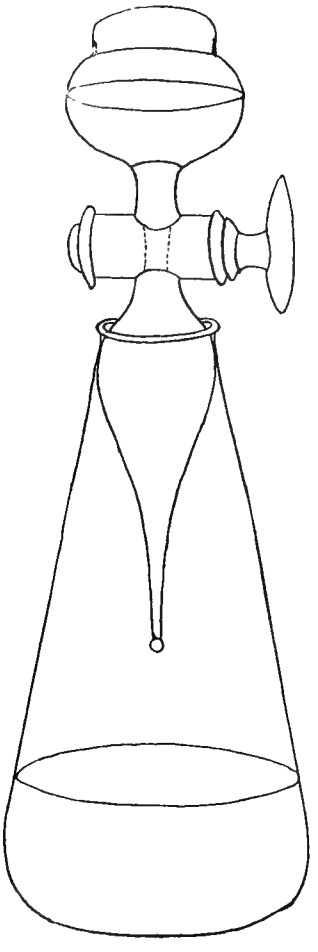
To convert		Grammes	into	Grains	×	15·432
„	„	„	„	ozs. Avoir.	×	0·03527
„	„	Kilogrammes	„	Pounds	×	2·2046
„	„	Grains	„	Grammes	×	0·0648
„	„	Avoir., ozs.	„	„	×	28·35
„	„	Troy, ozs.	„	„	×	31·104
„	„	Cubic centimetres (cc.)	„	Imperial fluid ozs.	×	0·0352
„	„	Litres	„	„ „ „	×	35·2
„	„	Fluid ozs.	„	Cubic centimetres (cc.)	×	28·42
„	„	Pints	„	Litres	×	0·568

28·42 cc. = 1 fluid ounce. 568·34 cc. = one pint. 1000 cc. = 35·196 fluid ozs.

Absolute alcohol of Commerce is generally 99·4 to 99·95%.

The sp. gr. of Ether used in the Celloidinizing process is	·720
„ „ of Chloroform used in the Paraffin and Celloidinizing process is	1·490—1·495
„ „ of the Oil of Cloves used in this work is	1·052
„ „ of the Oil of Cajeput used in this work is	·921
„ „ of the Oil of Bergamot used in this work is	·884

The term "Pure Benzol" used in this work signifies Rectified Benzol free from Thiophen.



Cap to oil cup.

Oil cup and narcotic carrier.

Regulating tap.

Graduating tube.

Oil drop.

Alcohol containing specimens.

A Graduating and Narcotising vessel, for graduating specimens from a light to a heavy fluid, and for narcotizing zoophytes, &c.

Clearing Oils.

Oil of Cajeput is recommended as a clearing agent for stained sections, because of its neutral action on the stains used.

Oil of Bergamot is used as a clearing agent for all celloidinized sections on account of it having no appreciable action on the celloidine. Celloidine is dissolved when placed in contact with oil of cloves or cajeput.

Oil of Cloves, being slower in its action than either of the above, is generally used for clearing tissue in bulk, and for graduating delicate objects out of alcohol, which would be contracted or destroyed by transferring them direct from alcohol to the oil (Fig. A).

Celloidine.

Schering's celloidine chips are recommended for use; it is put up in one ounce sealed boxes. Celloidine is soluble in a mixture of equal parts of absolute alcohol and ether, and is used for "celloidinising" animal and vegetable tissues, when it is required to bind or hold together any loose parts which we may desire to examine in their natural positions.

Celloidine does not "infiltrate" the tissues, but only surrounds and binds, or holds them in position during the manipulative processes.

Acidulated Alcohol.

Hydrochloric acid, two drops (minims).
92 per cent. alcohol, one ounce.

This solution is found useful in reducing the colour of overstained sections. Sections stained with carmine or hæmatoxylin may require a stronger solution for their reduction, or a prolonged soaking. Aniline-stained sections seldom require the use of acid alcohol, alcohol free from acid will generally be found sufficient for the purpose. Sections on which acid alcohol has been used must in all cases be rinsed in alcohol free from acid before passing them on to the next process.

Bleaching Solution.

Sections must never be bleached when it is desired to retain the cell-contents, but only when it is necessary to cleanse the sections of resinous matter so as to admit of better staining. After bleaching, the sections must in all cases be freed from the chlorine by prolonged washing in water, or by reagents, before staining.

CHAPTER I.

Collecting, Fixing, and Preservation of Specimens.

THE ultimate aim in microscopical research should be the acquisition of knowledge. Among the chief factors leading to this attainment are the methods adopted for collecting, fixing, and preservation of specimens to be operated on and studied. In the commencement of work let it be understood that every process a specimen is put through must be for the purpose of preparing it for the next process, and that every process must be carried out in its proper sequence from collecting the specimen to the finished microscopical slide. In all cases the specimens must be collected at the time and under the conditions best suited to the end for which they are intended. The phenomena of life, the cycle of life, from the single nascent cell from which it springs through all the manifold changes that take place in building up the individual plant or animal to its maturity, its reproduction, its decadence and disintegration, the ultimate separation of its elements and return to the earth and air from which it took its birth, should form the basis and goal of our investigations; and the importance of collecting the necessary materials at the proper time and under suitable conditions that these phenomena may be thoroughly examined, cannot be too strongly emphasised. It is necessary, therefore, that the student in practical microscopy should

have some knowledge of field and garden botany, and of natural history generally. Day by day we see plants spring from the earth, become bigger and bigger, and send out ramifications in every direction, but how few stop to study the phenomena of their growth; how does the plant or animal grow is the question that the microscopist has to answer; and herein lies the necessity for the use of chemical killing, fixing, and preservative agents in microscopical investigations, so that the dead tissues of plants and animals shall as nearly as possible present to the observer their natural conditions as when in a living state; thus enabling the investigator to trace out all the varied changes that take place during the life cycle of the plant or animal under observation.

Many methods have been devised for this purpose, among which the following have proved efficient, and will answer for the killing, fixing, and preservation of the specimens used to illustrate this work.

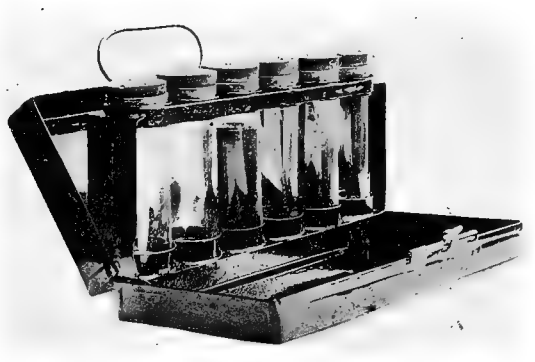


FIG. 1.—Collecting Case, containing six $3\frac{1}{2}$ in. × $1\frac{1}{4}$ in. tubes for fixatives.

The most simple preservative is methylated spirits of 92 per cent. in strength, into which may be placed immediately as collected, such specimens as roots, stems, leaves, leaf or flower-buds, ovaries, seeds,

&c., when it is intended only to show the position of the various parts of which such specimens are composed. By the action of the spirits on the specimens, resinous substances will be dissolved out, and the spirits become discoloured. The latter should now be poured off and the specimens covered with fresh spirits, in which they may remain indefinitely. For the fixation of cells, cell-contents, nuclear division, &c., of both plants and animals some specific fixative must be used, so as to fix and preserve them in their natural condition. As a general rule the reagents best suited for this purpose are:—

1. Osmic acid.
2. Chromic acid.
3. Acetic acid.
4. Picric acid.
5. Corrosive sublimate.
6. Formaldehyde.

These are combined to suit the purposes for which they are intended, the formulæ found most useful are made up as follows:—

1. *Flemming's Fluid.*

25 cc. of 1 per cent. chromic acid,
10 cc. of 1 per cent. glacial acetic acid,
55 cc. of water, and immediately before use add
10 cc. of 1 per cent osmic acid.

For use this solution should be taken into the field and immediately the specimens are collected they should be cut into as small pieces as possible compatible with the purpose for which they are required and placed directly into the solution, and allowed to remain there sufficiently long for it to completely penetrate the tissue. This will vary from one

to twenty-four or more hours, according to the size and density of the pieces dealt with. The specimens should now be soaked in water for four to six hours, if small, and for twenty-four or more hours, if large, to free them as much as possible from the acids, the water being changed at intervals during the process; or a much better method is to *tie up* the specimens in a muslin cloth, place them in a dish, and allow a stream of water to pass through them. The specimens should now be graduated through 25 per cent., 40 per cent., 60 per cent., 75 per cent., 85 per cent., and 92 per cent. alcohol, in which they may remain for future use. The specimens must stand in each strength of alcohol for one or two hours during the first few changes, and for eight to twelve hours during the latter two or three changes to prevent contraction of the tissues. The method of "graduating" applies in every case when it is necessary to transfer *delicate* specimens (or sections) from a dense to a light medium, or *vice versa*.

2. *Chromo-acetic Acid Solution.*

1 gram chromic acid, 1 cc. glacial acetic acid, 98 cc. water.	Stock solution.

This solution is much cheaper than Flemming's fluid, and may be kept ready for use in quantities suitable for one's requirements. It is the best all-round fixative for general purposes. The solution is a strong one, and should be weakened by the addition of water when it is desired to fix very delicate objects, or weaker solutions may be made up to suit individual requirements. In my own work it is used in its full strength for large pieces of tissue, developing vegetative buds, or male and female cones of pinus, vegetative buds and fertile spikes of

equisetum, &c. For flower or leaf buds take *two parts* of the solution and add one part of water, and for more delicate objects still (spirogyra) I take *equal parts* of the *solution* and *water* (Fig. 2).

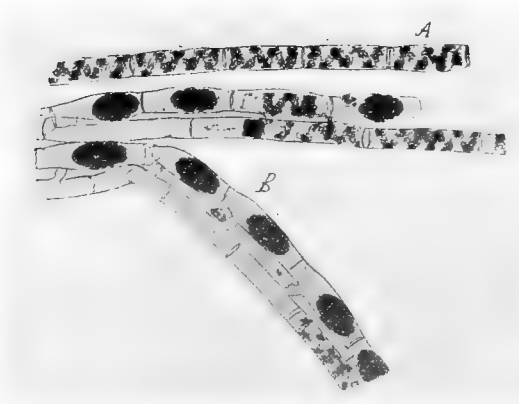


FIG. 2,—Spirogyra, fixed with equal parts of the chromo-acetic solution and water, magnified 80 dia. A, frond in normal vegetative condition; B, in conjugation.

This solution, with careful modifications, some knowledge relating to the nature of the objects under manipulation, and common sense, will serve as a fixative for, practically, the whole range of vegetable histology. After fixation the specimens must be treated as described above.

3. *Corrosive-picro-formaldehyde Solution.*

Boiling water, 100 cc.,
Corrosive sublimate, 2·5 grams, } Dissolve and add
Picric acid, 1 gram.

Allow to cool, and immediately before use add
Formaldehyde, 40 per cent. 10 cc.

This solution fixes vegetable and animal cells and their contents, perhaps, more completely than any known fixative. I have used it for

the fixation of developing pine buds, but have discarded it in favour of the chromo-acetic solution. It fixes everything so completely that the resinous substances in the cells, even after cutting the sections extremely thin, is so dark that the albuminous contents can scarcely be determined. After fixing, the specimens must be thoroughly washed, and afterwards graduated to 92 per cent. alcohol, in which they may remain for future use.

4. *Corrosive Sublimate.*

(A) A saturated solution in water.

(B) A saturated solution in 75 per cent. alcohol.

The water solution is used chiefly for the fixation of delicate specimens of the lower cryptogams. For use take 100 cc. of the solution and to this add 1 cc. glacial acetic acid. Specimens will fix in this solution in ten to fifteen minutes. After fixing, the specimens should be washed in several changes of water, and then graduated to 92 per cent. alcohol, when the nature of the specimen will allow this to be done without contraction of the tissue, in which case the specimens should be placed direct into a solution made up of eight parts distilled water and one part each of 92 per cent. alcohol and pure glycerine. This solution will serve also for permanently mounting the specimen.

When the alcoholic solution is used, which is applicable for general purposes, the specimens must be washed in several changes of 75 per cent. alcohol, to which a few drops of iodine solution, commercial, should be added, so as to liberate any corrosive that may have crystallised in the tissue, which if not removed may completely spoil the finished preparation by the appearance of metallic masses. When the iodine solution is added the alcohol takes on a brownish colour, which, however, soon clears, and if the addition of the iodine be

continued, the washing may be considered complete when the alcohol no longer clears, but retains the brown colour. The specimens may now be transferred to 92 per cent. alcohol, in which they may remain indefinitely.

5. *Picric Acid Solution.*

A saturated solution of picric acid in 92 per cent. alcohol.

This solution is used chiefly for fixing large pieces of tissue, its action being very rapid. The specimens must remain in the solution for several hours, or until the solution has completely penetrated through them; they must then be transferred to alcohol, which must be changed at intervals for a day or two to wash out the acid as far as possible. Vegetable tissue takes up picric acid very quickly and retains it so persistently that even after prolonged and repeated washings, it is scarcely possible to eradicate the acid until the tissue is sectionised, and the sections passed through two or three changes of alcohol.

6. *Formaldehyde.*

Formaldehyde, 40 per cent.,	3 cc.
Water	97 cc.

This is also made up in one to five per cent. solutions, according to requirements. A three per cent. solution being, perhaps, the most useful for all-round work. It is suited to most delicate objects, but is used more frequently in animal than in vegetable work. A two per cent. solution of formaldehyde may also be used as a mounting medium, but a stronger solution has a tendency to turn the objects opaque after they have been mounted for some weeks, and on this account I prefer carbolised water as a mountant.

The reason for the use of "fixatives" has already been stated. General rules only can be laid down for the guidance of students, the "absolute" is gained only by actual experience. Collecting specimens at the proper time and under suitable conditions, with a due regard to their fixation and after treatment, is of the greatest importance in histological work, as on this more than anything else depends the success of the after operations,—no after manipulative skill can rectify bad fixation and preservation. It is only after we have gained a knowledge of the nature of things that we can decide readily as to the use or non-use of fixatives, whether in any case their use is imperative, or altogether unnecessary. In all cases this will be indicated when dealing with the "type preparations" used to illustrate this work. The action of a true fixative is clearly illustrated by the photomicrograph Fig. 3 A, and an unsuitable one is shown at B. Both sections are prepared from the same specimen, one being fixed with the chromo-acetic solution, the other placed direct into 92 per cent. alcohol. The former is perfectly fixed, the cell-contents are preserved, and no contraction or shrinkage of the cell-walls has taken place. In the other there has been a complete breakdown and disorganisation of the entire system of cells and their contents, brought about by the rapid "dehydrating" action of the alcohol on the tissues. The specimen is an aquatic one, the cells of which are filled with water and the tissues are too delicate to resist the action of the alcohol, hence their collapse and destruction.

On the other hand, where the vascular system of the specimen is more highly developed, and when grown in dry soil, it may be plunged direct into 92 per cent. alcohol without any apparent injury to the tissues, as is clearly illustrated by the roots of Ranunculus and Zea mais (Plate 1, Figs. 1 and 4).

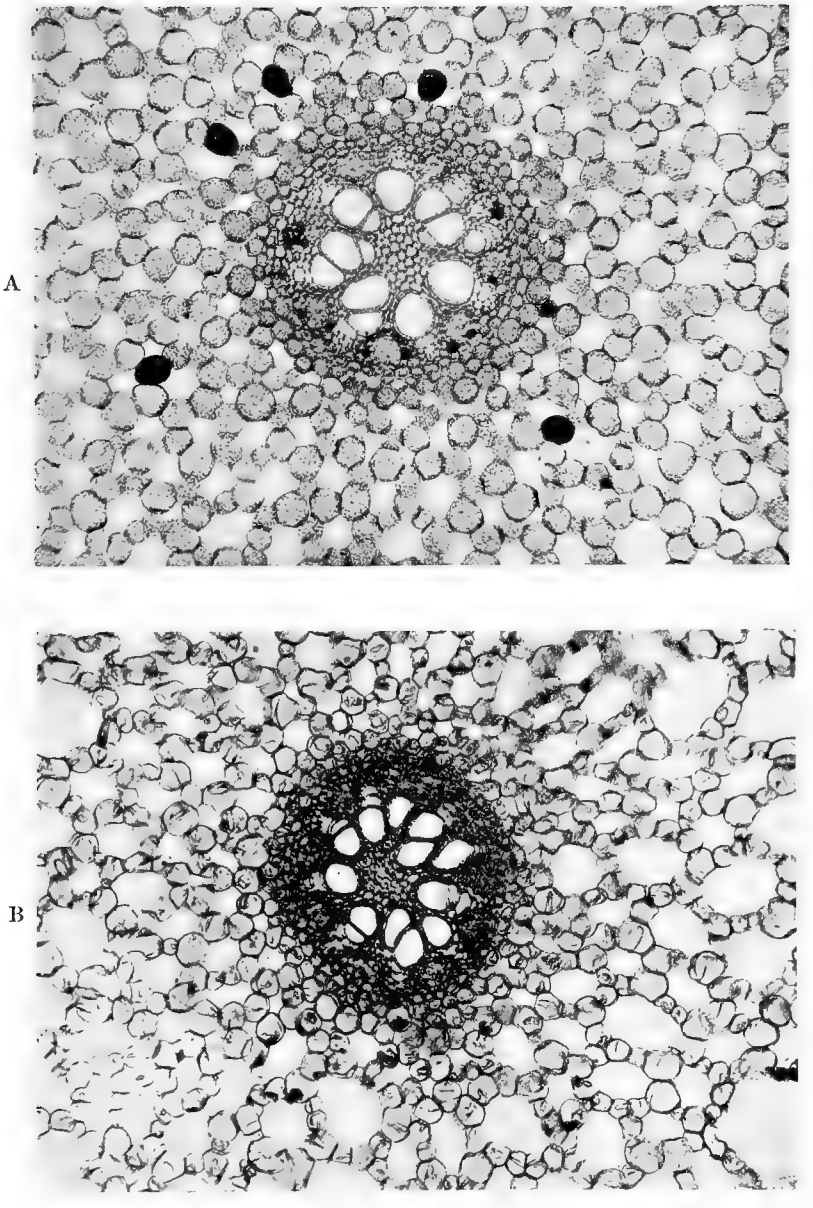


FIG. 3.—Transverse sections through root of "Acorus calamus" magnified 60 diam.
A. Fixed with chromo-acetic solution, and graduated to 92 per cent. alcohol.
B. Placed direct into 92 per cent. alcohol immediately when collected.

Chapter II.

Tools and Methods of Work.

The apparatus and instruments requisite for use in microscopical research vary in accordance with the requirements of each individual worker. The acquisition of a complete outfit for all-round research work is an expensive business, but much good work may be done with comparatively simple and inexpensive tools. In all cases the worker's

Fig. 4.—Tools required in the dissection of animals and plants.

outfit should be as simple as possible compatible with the work it is intended to undertake. The following list will be fairly complete for ordinary purposes, viz., a microscope with 1in., $\frac{1}{2}$in., and $\frac{1}{6}$in. objective,

microtome and section knife, paraffin bath and spirit lamp, turntable, forceps, needles, scissors, dishes and watch glasses, a graduated 100 cc. measure, pipettes, sable brushes of various sizes, wire clips, tin rings for building up cells, 3in. by 1in. plain slips, cavity slips, circular and oblong cover-slips, mounting block, stains, balsam, glycerine jelly, clearing-oils, bottles for specimens, incubator or drying apparatus.

Good microscopes are supplied by the leading opticians, but some difficulty may be experienced in obtaining a suitable microtome from this source, as each type appears to have been designed for one branch

FIG. 5.—The old "Flatters" microtome, from which the present microtome was evolved.

of work only, and that chiefly animal histology. I, therefore, describe somewhat fully the instrument I have used in my botanical and textile work since 1892. It is the outcome of several previous instruments, all of them more or less deficient in some respects. See Figs. 6 to 9.

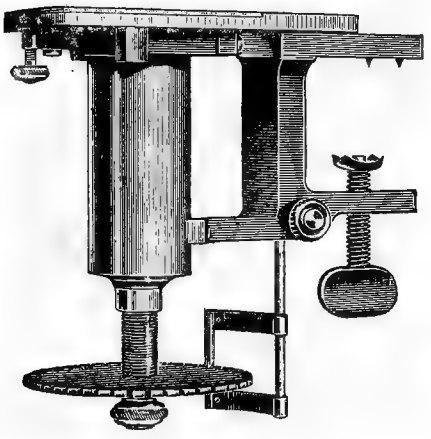


FIG. 6.—Showing Microtome ready for use.

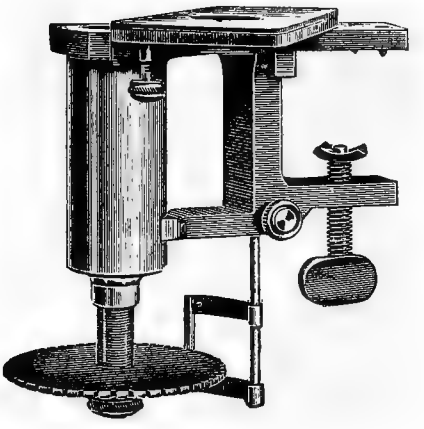


FIG. 7.—Showing knife-plate turned aside to allow the easy removal of the paraffin, after use.

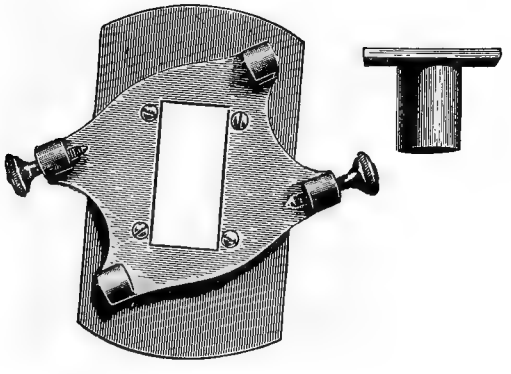


FIG. 8.—Showing underside of oblong top and side view of carrier of same.

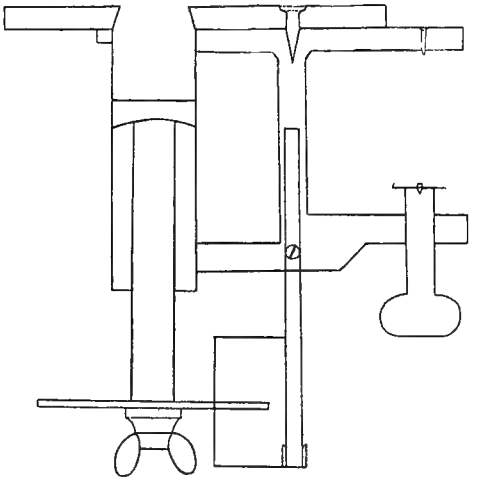


FIG. 9.—Vertical section

THE "FLATTERS" MICROTOME.

The microtome is made of brass, the tube or well is 3in. deep by 1in. in diameter internally, the spindle is of the same length, the screw having twenty-eight threads to the inch. The spindle is fitted with a thumb-screw at the lower end to admit of the toothed discs being easily changed. A spring stop, the tension of which can be adjusted, works in the teeth of the disc, thus ensuring a series of sections of uniform thickness. Three discs, divided as follow, are found to be sufficient for ordinary purposes, viz.:—

No. 1.	72 teeth,	giving sections	$\frac{1}{2000}$in.	in thickness	Approximately.
No. 2.	54 ,,	,,	$\frac{1}{1500}$in.	,,	
No. 3.	43 ,,	,,	$\frac{1}{1200}$in.	,,	

The latter being the one mostly employed for general purposes, and which when moved two notches gives sections $\frac{1}{600}$in. in thickness. Numbers 1 and 2 are used chiefly for the production of sections of animal tissue and of textile fibres. To ascertain the thickness of the sections obtained, multiply the notches in the disc used by the number of threads per inch on the spindle.

The knife plate, $2\frac{3}{8}$in by $4\frac{3}{4}$in., is made of hardened brass polished "dead flat," and has an aperture the same diameter as the tube, tapering slightly to the top to prevent the specimen from turning or rising while the sections are being cut; it is attached at one end to the headstock by a stout screw, and is securely held in position by a reliable catch, which is clamped under the headstock. The specimen to be cut is placed in the well of the microtome and melted paraffin wax, melting point 130 deg. F., poured in; this is allowed to set, and the superfluous wax is then removed. The "candle" so formed is then moved upwards by turning the toothed disc. The sections are cut by passing the knife

obliquely over the knife-plate, which is always kept moist with alcohol. The instrument is fitted with an oblong top, very useful for the production of sections from specimens which are too large for the ordinary well; it fits on top of the microtome (Fig. 10), and is held in position by a series of clamps. The aperture is $\frac{3}{4}$in. wide by $1\frac{1}{2}$in. long by $1\frac{1}{8}$in. deep, The carrier fits into the tube of the microtome and is actuated by the spindle in the usual way.

FIG. 10.—The Microtome, with the "oblong top" attachment set up for use.

The specimens to be cut are embedded in the well of the microtome in various ways according to their form and nature, the pieces to be cut must have one surface or edge levelled so that it will stand vertical on the carrier, and should be placed a little out of centre towards the cutting side, or operator, so that the knife has not to travel so far over the aperture before coming in contact with the specimen. As the specimen to be cut is surrounded with hard paraffin it is advisable to remove it from the front of the specimen, that the

knife may not be impeded by having to pass through it (Fig. 11). Very small objects that require some support to keep them in position during the time the paraffin is setting may be arranged on a piece of cork (which has been previously boiled in paraffin to expel the air) with a series of pins, these are withdrawn after the paraffin is set hard.

FIG. 11.—Well of Microtome, showing specimen embedded and paraffin removed from the cutting surface.

When there are a number of specimens to be sectionised, imbedding tubes, or moulds, Figs. 12 B may be employed; these should be about three-quarters of an inch high and of the same diameter as the well of the microtome, their inner surface must be smeared with glycerine to facilitate the removal of the blocks when the imbedding is complete,—these can be pushed out of the tubes and placed in the well of the microtome as required, a narrow groove must be cut longitudinally along the surface of each block to allow the air to escape from the well of the microtome, as it is being pushed in. A large series of imbeddings

may be made in this way, and the blocks placed in the stock-jars, where they can remain indefinitely. The specimens to be cut must in all cases be imbedded in the well of the microtome in such a manner that the section-knife shall pass through the exact zone of tissue required for examination.

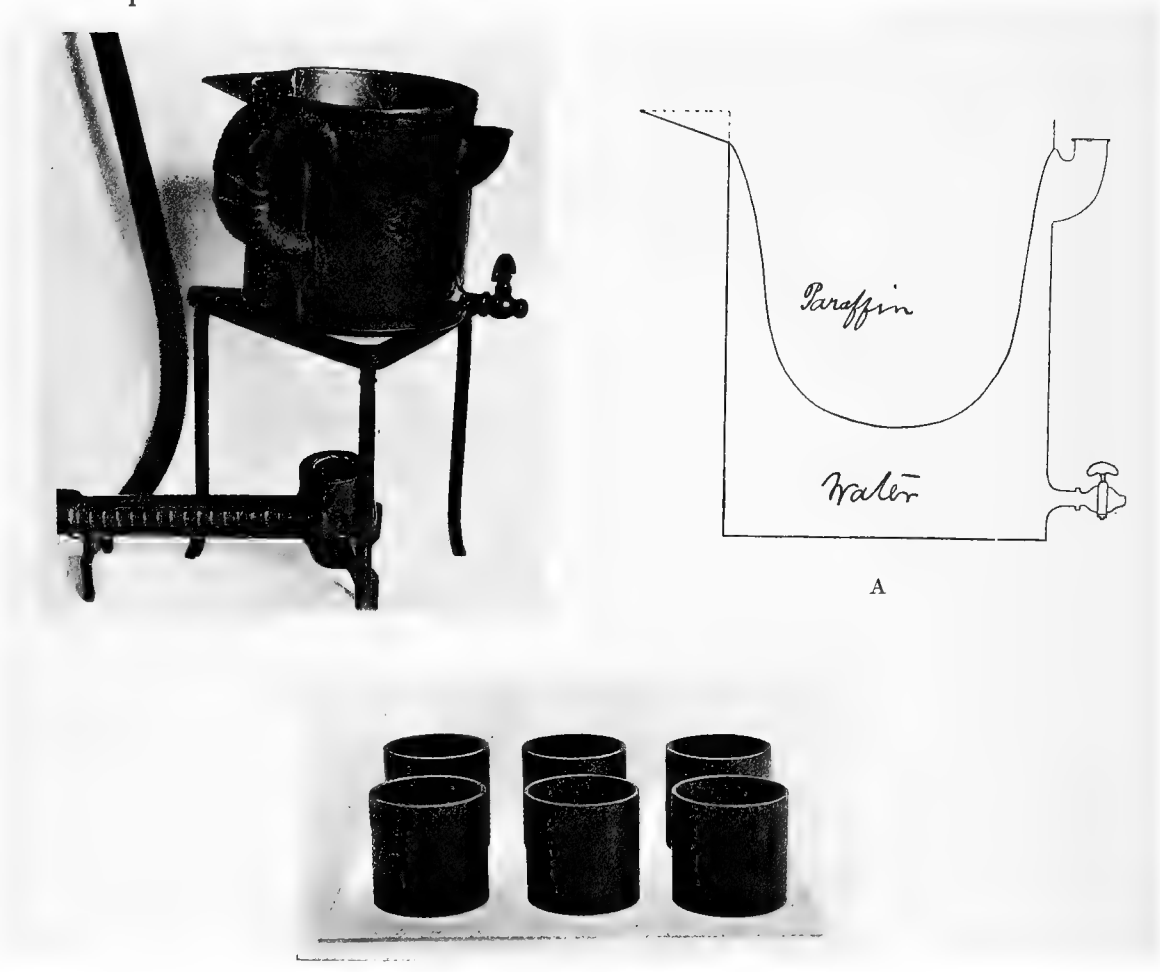
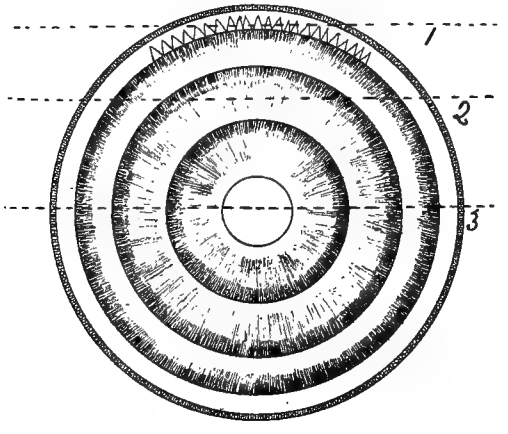


FIG. 12.—Paraffin bath with tripod and bunsen. A. Vertical section. B. Imbedding or moulding tubes.

The section-knife best suited for general use in botanical work is one having a straight handle with a fixed stout blade three to three and a half inches long, and slightly hollow-ground on both sides; the knife may be set on any ordinary stone, with the use of water and fine emery or carborundrum powder. A very small amount of this powder is placed on the stone with a few drops of water, and first rubbed into a very fine paste with an old disused section-knife. The knife for use is now set on this by passing it lightly over the stone *edge forward*, the traverse being from apex to base, and base to apex. The back and edge of the knife must be parallel to each other, and in true line from back to edge.

The Art of Cutting Sections.

To make a complete study of a solid mass of tissue, sections must be cut at different planes to each other, viz., "transverse," "radial-longitudinally," and "tangential-longitudinally." The transverse section



FIG, 13.—Diagram of dicotyledonous stem. 1. Tangenital area passing through the bast fibres. 2. Tangenital area, passing through the xylem. 3. Radial line, passing through the pith.

is taken at *right angles* to the *axis* of growth. The radial longitudinal section is *taken through* the *axis* of growth. The tangential longitudinal section may be *varied* in its direction between the central *axis* and the *circumference* in accordance with *position* of the *special tissue* it is desired to examine. Each section when placed under the microscope will present to the observer a surface cut at right angles to each other, and without which a complete study of the tissue could not be made. It is, therefore, of the greatest importance that the student should

A B

FIG. 14.—Photographs of the operator in the act of cutting sections. A, showing the point of the knife in contact with the specimen. B, showing the position of knife at completion of cut, with the section resting on the blade and the paraffin curled over in front of it. The movement of the cut is a sliding one, its traverse being from right to left, the full length of the blade having been used in the operation.

thoroughly understand the principle of section-cutting, as on this, more than anything else, depends his success or failure in the production of satisfactory preparations.

The melting point of the paraffin used for imbedding purposes is 130 degs. Fahr. When new it has a tendency to "crumble" when the

sections are being cut, but its consistency may be modified by the addition of a little vaseline, and is much improved by constant and repeated use. There is no other medium required for imbedding ordinary roots and stems, and many of the leaves, seeds, &c., even the cellular aquatic roots and stems are imbedded for cutting in this way. The specimen is taken out of the stock jar, placed in position in the well of the microtome, and held in position with a needle while the melted paraffin is being poured in. After the "candle" so formed is set hard, the superfluous paraffin is removed from the knife-plate with an old section knife, which completes the operations preparatory to section-cutting.

Preparing Specimens for Sectionising.

Most of the delicate tissues and all specimens having loose parts require some special method of treatment before they can be sectionised with success. The methods best adapted for this purpose are the

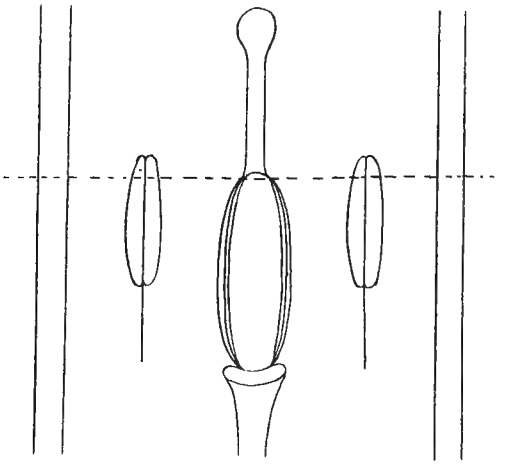


FIG. 15.—Diagram showing the various positions of floral parts in a lily, the dotted line being the line of cut for celloidinising purposes.

"paraffin" and the "celloidinising" processes. Under treatment with paraffin, the tissues of plants and animals become infiltrated and form a solid block.

With the celloidinising process the *parts* of the specimens only are surrounded, held in position and supported during the sectionising and succeeding manipulations, the celloidine adhering to the section when cut is treated as being a part of the section itself, and is mounted as such on the finished slide.

On the other hand paraffinised sections are fixed on the slide with albumen and the paraffin dissolved out. Specimens it is intended to celloidinise must have their parts so exposed as to admit of the access of the celloidine, and to facilitate this operation the *apex* of anthers, ovaries, leaf and flower buds are cut away, the cut being sufficiently low to expose all the parts it is intended to keep *in situ* when the sections are taken (Fig. 15).

The Celloidinising Methoa.

1. Place the specimens in absolute alcohol for twelve to twenty-four hours, according to size and density of specimens; if they are large the alcohol should be changed once or twice. It is important that the specimens should be thoroughly dehydrated before passing them on to the next process.

2. Place the specimens in ether, allow to stand twelve hours; change as above, if necessary.

3. Place the specimens in equal parts of *Absolute alcohol* and *ether*, to which add half an inch of Schering's celloidine chips, this will *dissolve* in four to six hours, and *penetrate* the *interstices* of the *specimen*. A little of the celloidine chips must be added day by day until the *solution* becomes sufficiently *concentrated* to form a

jelly-like mass; each addition of celloidine must be *thoroughly dissolved* before the next is added, stir or shake occasionally to facilitate the process.

4. Transfer the specimens from the mass with *fine-pointed* forceps and drop them one by one into *chloroform.* There must be *sufficient* celloidine adhering to the specimens to form a *protective coat.* The action of the chloroform is to *coagulate* or *solidify* the *celloidine* and form a *solid block.* At first the specimens will *float* on the surface of the chloroform, but as they become solidified they will *sink* to the bottom of the vessel; the process will be complete in six to eight hours, when any surplus celloidine adhering to the specimens may be removed, and the specimens placed in 92 per cent. alcohol, where they may remain indefinitely.

In actual practice I find it preferable to use two parts of ether and one part of absolute alcohol, rather than equal parts of each,—the specimen works out more solid and is better to cut, celloidine also has a less tendency to take up the stains. The specimens are imbedded in the well of the microtome with melted paraffin, in exactly the same way as the uncelloidinised specimens; the sections are placed direct into 92 per cent. alcohol, from which they may be stained, then cleared with oil of *bergamot,* and transferred to a *thin solution* of *balsam* and *benzol,* out of which they may be mounted when convenient.

The reason for the use of oil of *bergamot,* in clearing celloidinised sections, is that it has no *appreciable* action on the celloidine, whereas oil of *cajeput* and oil of *cloves dissolve* the *celloidine,* and by their use the sections would be destroyed.

To Infiltrate Specimens with Paraffin.

Specimens of either vegetable or animal tissue which cannot be cut sufficiently thin by the celloidinising method and by means of the

ordinary microtome must be infiltrated with paraffin, and cut by means of some mechanical arrangement. The "Cambridge Rocking Microtome" is well suited for this purpose, its traverse movement being actuated to $\frac{1}{40000}$ of an inch, and by its use sections have been obtained in my laboratory of animal tissue (suprarenal gland of Cat) cut to $\frac{1}{15000}$, $\frac{1}{30000}$, and $\frac{1}{100000}$ of an inch in thickness for "test" purposes for Mr. J. E. Storey, of the Manchester Microscopical Society. For ordinary purposes sections seldom require cutting thinner than $\frac{1}{5000}$ of an inch in thickness.

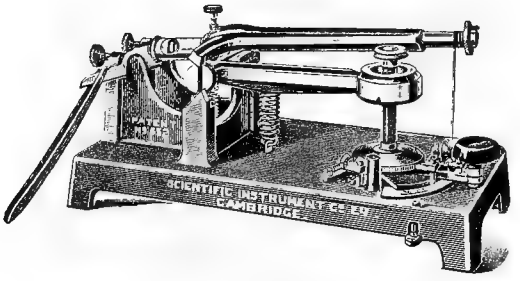


FIG. 16.—The Cambridge "Rocking" Microtome.

The primary method of infiltrating vegetable tissue with paraffin is the "Chloroform" or the "Xylol" process, either of which has but little injurious effect on the tissue if properly conducted. I give here full details for the Chloroform method, but Xylol may be substituted for chloroform by those who prefer it:—

1. Transfer the specimens from 92 per cent. into *Absolute alcohol*, allow to stand twelve to twenty-four hours according to size and density of specimen, change the alcohol once or twice during the time.

2. Place the specimens in equal parts of *Absolute alcohol* and *chloroform*, and allow to stand twelve hours.

3. Place the specimens in *chloroform*; allow to stand twelve hours.

4. Place the specimens in a *saturated* solution of *paraffin* in *chloroform;* allow to stand twelve to twenty-four hours or longer according to size of specimen. Melting point of the paraffin should be about 130 degs. Fahr.

5. Transfer the specimens to paraffin (free from chloroform) melted in the water-oven—the heat must not be higher than one degree above the melting point of the paraffin; allow the specimens to remain in the water-oven for two or three hours, change the paraffin at intervals. By this means the chloroform is evaporated, or thrown off, and the tissues become infiltrated with pure paraffin. It would perhaps be preferable to use several tubes containing melted paraffin and transfer the

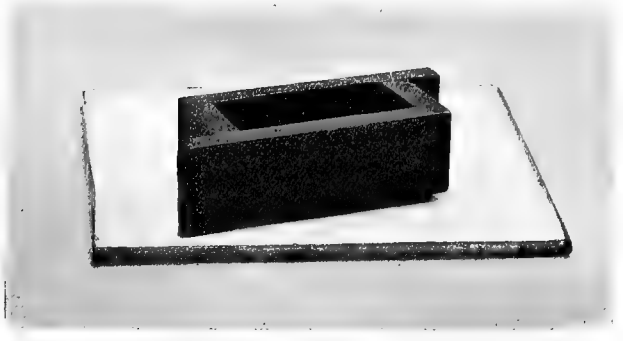


FIG. 17.—Moulding frame, formed of two L-shaped pieces of brass, with arms one and a half inches long and half-inch angles.

specimens from one to the other, allowing them to stand a short time in each, care being taken that the heat is only just sufficient to keep the paraffin in a fluid condition.

6. Have ready a quantity of freshly-melted paraffin, pour this into the mould (Fig. 17), place in the specimen, and arrange it in

position for cutting, with hot needles, and as soon as the "block," or mould, is just set on the upper surface plunge the entire mould into cold water; by this means the block will be cooled uniformly. If this operation be carried out successfully the block, when removed from the mould, will appear uniformly transparent. Should this not be the case one of three things will have taken place:—

(1) The specimen has not been properly dehydrated;

(2) The chloroform has not been completely evaporated;

(3) The block may have been cooled too slowly.

Either defect will be indicated by a milky opacity in the centre of the block surrounding the specimen; the only remedy is to re-melt the paraffin and re-mould.

7. The block containing the specimen should now be trimmed into a square; warm the paraffin in the orientator, or carrier of the microtome, and also the base of the block containing the specimen, press them gently together; then seal the joint with a hot needle, and the preparation is ready for sectionizing. After the sections are cut they may be fastened to the slide with Mayer's albumen solution.

Formula.

White of egg, 50 cc.
Pure glycerine, 50 cc.
Salycilate of soda, 1 grain } Shake well to dissolve. Filter for use

Place a drop of the solution on the slide and smear it evenly over the surface, and then wipe as much off as possible either with the hand or with a clean piece of non-linty cloth. Now place a few drops of water on the slide, or float the section on to it, warm gently over the spirit lamp, when the paraffin and the section will become smoothed out and free from wrinkles. Now tilt the slide and allow the water to drain off, guiding the sections into position with a needle while doing so. The slide should now be placed in the warm incubator for an hour or two to dry. The paraffin may now be removed by gently heating the slide over the spirit lamp to the melting point of the paraffin, and placing direct into xylol, which completes the operation by dissolving out all traces of paraffin, and by the heating operation the albumen will be coagulated, and the section cemented on the slide.

The slide with the sections should now be rinsed in one or two changes of alcohol and it is ready for staining. After staining, place the slide in xylol for ten minutes to clarify the section, drain off the surplus xylol, and mount the section in "xylol balsam."

Staining.

The art of staining consists in differentiating the various tissues of which plants and animals are composed, the operations being governed by the chemical affinities of the tissues to be stained, and these vary considerably in accordance with the age of the tissues themselves. No tissue will take up a stain unless there is an affinity between the tissue and the stain, and no simple tissue will take up two stains unless the cells composing it are nucleated. A preparation composed of two or more tissues may be stained in two or more colours, but two or more colours cannot be put into a preparation unless there is a tissue for each of them, for example, developing vegetable tissue may be differentiated

to the extent of half a cell-wall, the juncture of the cells first showing a slight lignification, and having an affinity for a stain giving a lignin reaction, while the central portion of the cell-wall is still unlignified and takes up a stain having an affinity for cellulose; each stain gradually merging into the other and in each case showing the deepest in colour at that particular part having the greatest affinity for it.

The production of a correctly stained section does not consist so much in putting the stain into the tissues, as in washing it out; the finest preparations being those that have been overstained rather than otherwise and the colour stripped out again, or reduced to the required tint by the use of acid-alcohol or by a prolonged soaking in alcohol. When a nuclear preparation only is required, the reducing operation must be continued until the colour is removed from all the tissues except the nucleus. When it is desired to double stain a preparation composed of two or more tissues, it must be placed first into the stain having an affinity for the primary ground tissue, and treated exactly as if staining only for one colour, and then placed in the second or counter-stain. After staining with the second colour, the sections should be rinsed quickly in two or three changes of alcohol, and immediately the first colour stands out clearly the process must be stopped by transferring the sections to the clearing oil.

As a simple example of staining, let us take *Grenacher's* formula of borax carmine, which is practically a permanent stain for developing tissue. To remove this stain from tissue for which it has an affinity requires the use of acidulated alcohol, or to be left for weeks in alcohol free from acid, or placed direct from the alcohol into water when diffusion currents would be set up and the carmine liberated.

On the other hand Malachite green, which is a counter stain to carmine, having an affinity for formed or lignified tissue, may be

removed from the tissue by a comparatively short washing in alcohol without the aid of acids; it follows, therefore, that in all cases the sections must be stained first with the primary or most permanent colour, and secondly with the counter-stain, out of which they must be rinsed quickly and transferred to the clearing oil as described above.

Protoplasmic and nucleated tissue takes up stains more readily than formed or dead tissue. A diffuse stain affects all the elements of a preparation, while a specific stain affects only certain elements, or stains some elements more deeply than others. A nuclear stain is one which has a strong affinity for the nucleus, and which is retained by it when all the surrounding tissue is freed from colour.

Grenacher's formula of borax carmine and Kleinenberg's formula of Hæmatoxylin are diffuse stains; whereas on the other hand Brazilin and Delafield's formula of Hæmatoxylin are decidedly nuclear and specific stains, and for permanent work are perhaps the most useful stains known to us, with Safranin, Gossypimine, and Malachite green as counter stains.

Staining will vary in accordance with the nature of the tissue dealt with and its prior treatment, if, for example, we prepare sections from a lateral branch, one eighth of an inch in diameter, of the Lime tree, collected at the end of March when the tree with all its ramifications is infused with new life, they will take up the stains much more readily and retain them much more persistently than if collected and prepared in December when the tree is in a more or less dormant state.

Mounting and Finishing the Slide.

After the sections have been stained and cleared, they should be allowed to stand for a short time in a thin mixture of Benzol balsam;

this not only hardens the sections, but also frees them from the clearing oil, which, if not removed before the sections are permanently mounted, diffuses out afterwards and discolours the balsam; a common cause of the dirty brown appearance of so many commercial slides. The sections may remain in the benzol balsam indefinitely, if needs be, without deterioration in colour, as in this condition they are practically mounted. In my own work I have sometimes a dozen of these "balsam-pots," each containing half a gross of sections waiting for days

A. B.

FIG. 18.—Complete staining and slide-mounting outfit. A. Storing cabinet. B. Microscope, mounting blocks, section dish, drying tray, &c.

and in some cases weeks before they are permanently mounted, the rims are smeared with balsam and a glass cover placed on them, which practically seals them protecting the sections from dust, and the benzol from evaporation. The sections are mounted permanently by placing a drop of ordinary Benzol balsam on the centre of a plain slip, the section is lifted out of the thin benzol-balsam and placed in this; and then a cover slip is placed on the section, the whole being clamped in

position by means of the wire "spring-clip" (Fig. 18 B), until the slide is baked, or has set hard by the evaporation of the benzol. In mounting solid specimens on cavity slips, the cavity must first be filled with balsam, the specimen placed in this, and then a cover slip, whether round or oval, one size larger than the cavity is placed in position. In this case the spring-clip must be applied with only just sufficient pressure to keep the cover in position during the drying operation, or

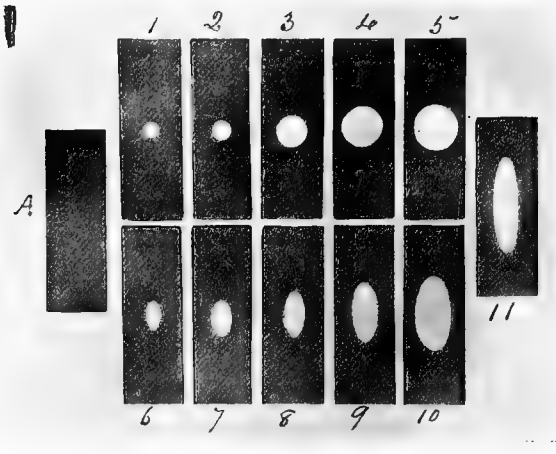
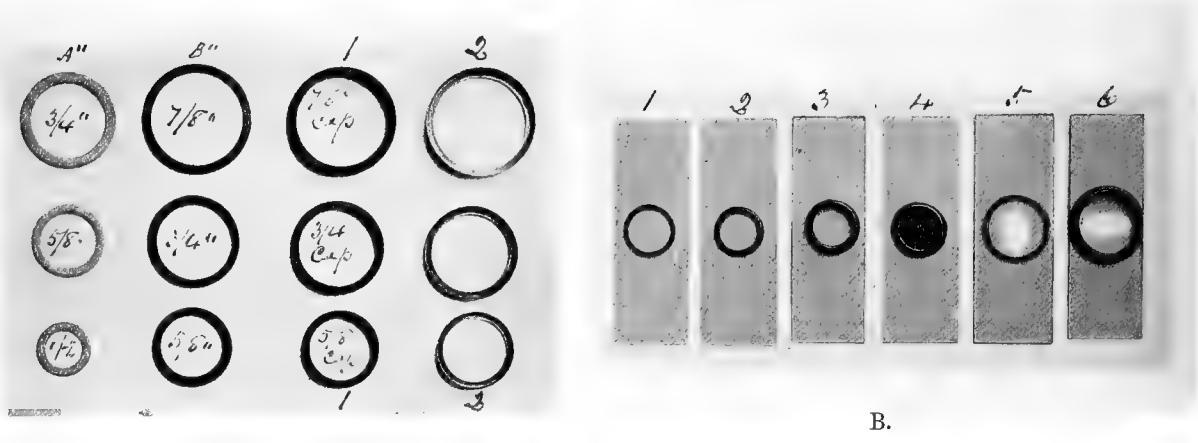


FIG. 19.—Types of 3in. by 1in. slips, used for mounting microscopical objects.
A. Plain slip. 1-5. Round cavity slips. 6-11. Oval cavity slips.

there is a danger of the cover being depressed, by which means some of the balsam would be squeezed out, and when the clip was removed the cover would again spring up, causing suction thereby and consequent air-bubbles to find their way into the cavity.

When it is required to mount large entire specimens in fluid and the ordinary cavity-slip is not suitable to this requirement, a cell should be built up round the cavity with tin rings.

The slip must be placed on the turntable, and a ring of brown cement run round the cavity (Fig. 20, B, 5-6); this must be allowed to set hard, then another layer of the cement should be applied on the top, into which the tin ring must be imbedded, and another cement ring to seal it completely to the first. This must be allowed to dry thoroughly, and, if necessary. the process repeated until the cell is built up to the required height, so that the specimen can be enclosed without having to use pressure upon it. A light "*tacky*" ring of brown cement should now



A. B.

FIG. 20.—A. Rings for building up cells. A". Cardboard rings made out of old post cards, used for dry mounting. B". Tin rings for balsam or fluid mounts. 1. Upper side of caps for same. 2. Underside of caps showing the flange. B, 1-6. Cells at various stages of construction. 4. With black background for opaque mount. 5 and 6. Cells surrounding a cavity to give depth.

be applied to the *top* of the cell, the cell filled with the mounting fluid, and the specimen arranged in position, (care being taken that the fluid stands slightly above the margin of the cell), the cover slip must now be applied to the left margin of the cell and gently lowered into position, at the same time flushing out the superfluous fluid. Now place the mount on the turntable, which is set in motion, and a little pressure applied to

edge of the cover slip with the forceps to imbed it firmly into the "*tacky*" ring (Fig. 21, A and B). If this be done carefully no difficulty will be experienced with air bubbles. A little of the fluid may have been pressed out of the cell by this operation, this must be absorbed with filter paper, and a fresh ring of cement applied to seal up the junction of cover and cell. The preparation must now be allowed to dry

A. B.

FIG. 21.—A. Method of placing cover slip on a fluid mount. B. Turntable in action and cover slip being pressed into position. C. Mounting plate on turntable carrier.

thoroughly for some days, when it should be rung with old gold size, and while this is still moist a protective cap (Fig. 20, A, 1-2) must be placed over the top of the cell, and a fresh stout ring of gold size applied over the entire structure, and the preparation again put away to dry. A ring of "zinc oxide" made with the proper proportion of "old gold size" may now be applied; this must *over-lap* all the previous rings, and if the cements be properly made and properly applied the preparation will stand for a lifetime.

The method of building up a cell for a balsam mount is much more simple than the above. Balsam is placed in the cavity; then the specimen is arranged in position, a tin ring is cut across and each half arranged round the cavity with a small space left between them (Fig. 22). A cover slip is placed on the ring and the whole held in position by a spring clip. If the cell is not already full of balsam more may be run under the cover slip through one of the openings in the ring. The preparation must be allowed to dry *slowly*; and after the superfluous balsam is removed, the cell should be protected with several layers of brown cement, and ultimately finished off with zinc oxide.

FIG. 22.—Tin ring divided for building up balsam mounts.

Cleaning, Ringing, and Finishing the Slides.

Balsam preparations are dried by being placed in a water-oven for sixteen hours at a temperature of 140 degs. Fahr., or in the absence of the water-oven, should be put aside in a warm place for a few days to evaporate the benzol and set the balsam. If the water-oven be used for this work, the slides, after drying, must remain in it until it has gradually cooled down; they should then be taken out, the spring clips removed, and the superfluous balsam cleaned off with the "hot-knife." The hot-knife may be made out of an old table-knife by reducing the point end to a chisel edge, which must be square. This is heated in the bunsen flame and then lightly passed round the edge of the cover slip carrying the balsam with it (Fig. 23 A).

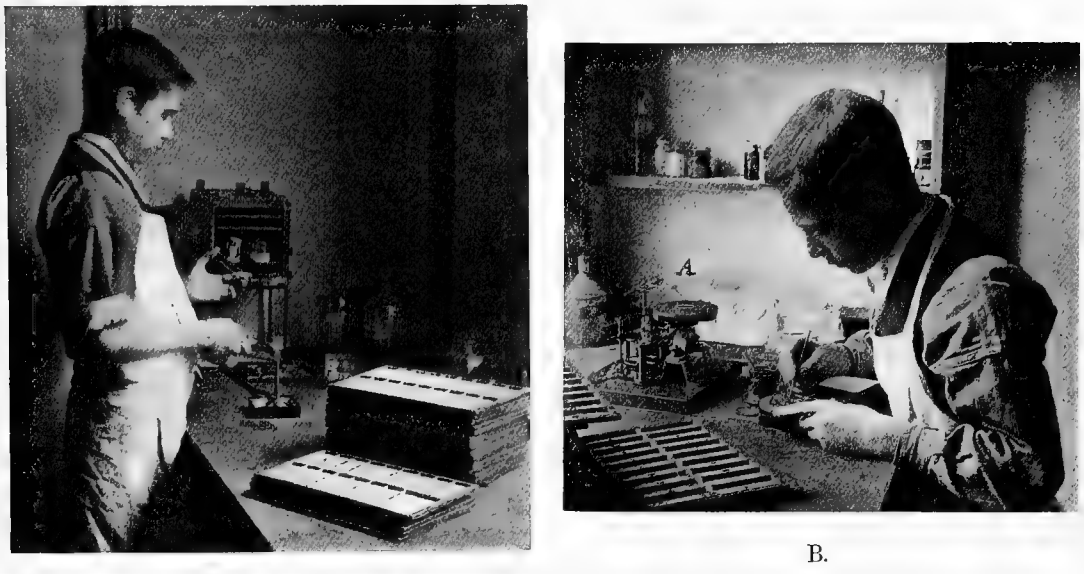


FIG. 23.—Hot-knifing and ringing slides. A. Hot-knifing. B. Putting on the first cement ring.

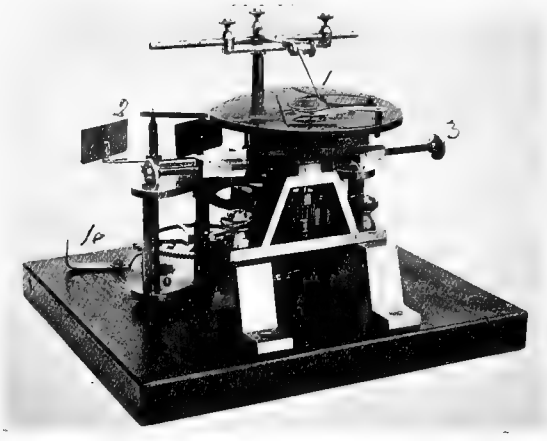


FIG. 24.—Turntable for ringing ovals, run by clockwork. 1. Guiding pointer, under which the brush is held when ringing. 2. Governor plates of clock. 3. Decentralising motion. 4. Stop-catch.

The smears left by this operation may be removed with a soft cloth moistened with 92 per cent. alcohol. The superfluous jelly, on glycerine jelly mounts, may be removed by washing the slide in cold water with a stiff tooth brush. The slide should now be placed on the turntable and the preparation sealed up with a brown cement ring, as already described. When the brown cement is dry, a ring of zinc oxide may be applied, and on the top of this a finishing ring of asphaltum, or a single black finishing ring may be applied over the brown cement one; the slide should now be labelled and named for use.

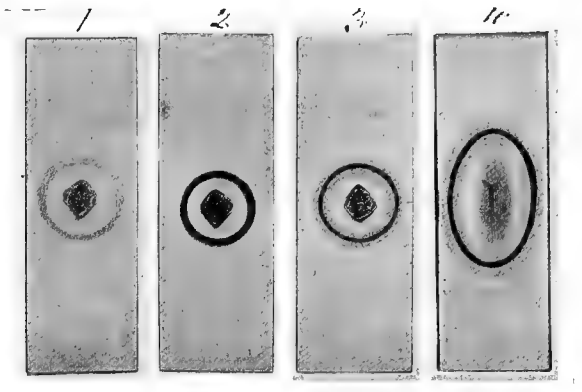


FIG. 25.—Slides in various stages of cleaning and ringing. 1. Before hot-knifing. 2. First ring of brown cement. 3. Finished with zinc oxide and asphaltum. 4. Oval mount, finished same as number 3.

Chapter III.

Stains, Reagents, Mounting-media, Cements, etc., their Formulæ and Manipulation.

The following formula and manipulative methods are those daily practised by me in the production of animal and vegetable preparations. The list is a very limited one, but will be found sufficiently extensive to cover the requirements of the general worker. The specialist may extend the formulæ and methods to meet his own wants.

No. 1. BORAX CARMINE.—Grenacher.

Best carmine	5 grams.
Borax	8 grams.
Water	480 cc.
Alcohol 92%	480 cc.

Dissolve the carmine and the borax in the water by heat.

When cold add the alcohol; allow to stand a few days to ripen; filter for use.

Manipulation.

1. Pour off the alcohol and cover the sections with the stain, and allow to stand fifteen to thirty minutes.

2. Pour off the stain and rinse the sections with *acidulated alcohol* (two drops of Hydrochloric acid to one ounce or 30 cc. of 92% alcohol); then rinse with alcohol free from acid. Now allow the sections to stand in alcohol for thirty minutes, changing the alcohol during the interval if necessary.

3. Pour off the alcohol and cover the sections with oil of cajeput, and allow to stand fifteen minutes to clarify.

4. Transfer the sections to thin balsam and benzol, and when convenient mount the sections in balsam.

The use of hydrochloric acid in process 2 is to remove the surplus stain out of the sections, and if the sections are thick and deeply stained the acid wash must be prolonged until the colour is reduced to the required tint. The acid should never be used if the colour of the section can be reduced to the proper tint by the use of alcohol alone. Two drops of acid to one ounce of alcohol is a useful standard to work with, but in many cases six to eight drops to the ounce will be required; on the other hand the merest trace of acid will remove some stains completely from the sections. Carmine and Hæmatoxylin are the two chief stains for which the acid should be used; nearly all other stains may be reduced to the required tint by the use of alcohol alone.

No. 2. **BORAX CARMINE.—Woodward.**

Best carmine	3 grams.
Borax	12 grams.
Water	480 cc.
Alcohol 92%	480 cc.

Mix the ingredients and filter for use. *The first filtrate.*

The crystals left on the filter paper must now be washed into 960 cc. of water, dissolve by heating to boiling point; when cold filter for use. *The second filtrate.*

The first filtrate is a weak stain and slow in its action, but it is specially useful for staining entire specimens such as pinnæ of ferns, spikes of selaginella, etc., and is specially applicable for staining marine zoological specimens.

The second filtrate is more rapid in its action, and is useful for staining sections or specimens that are to be mounted in glycerine jelly; the colour being practically permanent in that medium.

MANIPULATION FOR THE FIRST FILTRATE.

1. Pour off the alcohol and cover the specimens with the stain, and allow to stand from one to six or eight hours, according to the size and density of the

specimen, which must be examined at intervals, to ascertain the progress of staining, and when deep enough in colour, the specimens should be rinsed in two or three changes of alcohol.

2. Place the specimen in *Absolute alcohol* and allow to stand for twelve hours to thoroughly dehydrate them, and if they are large change the alcohol once or twice during the interval.

3. Pour off the alcohol and cover the specimens with oil of cloves, allow to stand until clarified.

4. Transfer the specimens into thin balsam and benzol, and allow to stand for several hours to free them from the oil of cloves.

5. Mount the specimens in balsam in cavity slips, without pressure.

MANIPULATION FOR THE SECOND FILTRATE.

Specimens or sections to be stained from water.

1. Pour off the water and cover the specimens or sections with the stain, allow to stand—in the case of *sections*, for fifteen to thirty minutes,—in the case of specimens, one to three hours according to their size and density. Examine the *specimens* from time to time, and when deep enough in colour, rinse in two or three changes of 25% alcohol.

If the specimens are to be mounted in balsam they must be graduated to 92% alcohol, and then placed for several hours in absolute alcohol to thoroughly dehydrate, and treated for mounting as described above for the first filtrate.

If the specimens are too delicate to admit of mounting in balsam, prepare them as follows for mounting in glycerine jelly. After staining and dehydrating, place the specimens, first, into one part pure glycerine and two parts 92% alcohol; secondly, into equal parts of glycerine and alcohol; thirdly, into pure glycerine; allow the specimens to stand an hour or two in each.

2. Mount the specimens in glycerine jelly.

No. 3. HÆMATOXYLIN.—Kleinenberg (modified).

Hæmatoxylin	1 gram.
Calcium chloride	8 grams, in 40 cc. of water.
Alum	3 grams, in 60 cc. of water.
Alcohol 92%	770 cc.

Dissolve the alum and the calcium chloride in their respective quantities of water by heat.

Mix the two solutions, and immediately add 700 cc. of the alcohol slowly; allow to stand for an hour; filter.

Add the hæmatoxylin dissolved in the remaining 70 cc. of alcohol, and place the bottle in the sunlight for a few days to ripen; filter for use.

MANIPULATION.

1. Pour off the alcohol and cover the sections with the stain, and allow to stand fifteen to thirty minutes.
2. Pour off the stain and rinse with accidulated alcohol, then with alcohol free from acid.
3. Pour off the alcohol and cover the sections with oil of cajeput, and allow to stand fifteen minutes to clarify.
4. Transfer the sections to thin balsam and benzol.
5. Mount the sections in balsam.

No. 4. HÆMATOXYLIN.—Delafield.

NUCLEAR STAIN FOR VEGETABLE AND ANIMAL TISSUES.

200 cc. of a saturated solution of ammonia alum.

2 grams of hæmatoxylin in 12 cc. absolute alcohol.

Mix the two solutions, and expose to light and air for a week. Filter, and add to the filtrate.

50 cc. pure glycerine.

50 cc. of methylic alcohol (wood spirits).

Allow the solution to stand in the sunlight until the colour develops. Refilter for use.

MANIPULATION.

Sections to be stained from water.

1. Pour off the water and cover the sections with the stain, allow to stand ten to thirty minutes according to requirements, or nature of the tissue under treatment.

2. Pour off the stain, and rinse the sections in two or three changes of 25% alcohol, then graduate to 92% alcohol.

If the sections are overstained, or if the staining be too diffuse, treat them with the acidulated alcohol until the stain is removed from all parts except the nucleus. The staining may be made more precise in its action if the stain be diluted with several times its own volume of water, and the time in staining be prolonged accordingly.

3. Clear the sections, if uncelloidinized, in oil of cajeput.

4. Transfer the sections to thin balsam and benzol, and when convenient,

5. Mount the sections in balsam.

No. 5. AMMONIATED HÆMATOXYLIN.—Ehrlich.

NUCLEAR STAIN FOR VEGETABLE AND ANIMAL TISSUES.

Hæmatoxylin	15 grams.
Ammonium carbonate	15 grams.
Absolute alcohol	300 cc.

Place in a large bottle and shake occasionally for three days, leaving the stopper out during the intervals.

Place the solution in an open dish, and allow it to evaporate to dryness at the temperature of the air.

Dissolve the crystalline product obtained in

Alcohol 92%	750 cc.
Pure glycerine	750 cc.
Pure water	750 cc.
Glacial acetic acid...	75 cc.
Ammonia alum	15 grams.

Filter for use.

MANIPULATION.

Sections to be stained from 25% alcohol.

1. Pour off the alcohol and cover the sections with the stain, allow to stand fifteen to thirty minutes.

2. Pour off the stain and rinse the sections in two or three changes of 25% alcohol, and graduate to 92%. Reduce the colour if necessary with acidulated alcohol.

3. Pour off the alcohol and cover the sections, if uncelloidinized, with oil of cajeput; allow to stand ten to fifteen minutes to clarify.

4. Transfer the sections to thin balsam and benzol, and when convenient

5. Mount the sections in balsam.

No. 6. BRAZILIN.

NUCLEAR STAIN FOR ANIMAL AND VEGETABLE TISSUES.

A. The mordant.

Iron Alum	1 gram.
Alcohol 75%	100 cc.

Dissolve and filter for use.

B. The stain.

Brazalian crystals	1 gram.
Alcohol 75%	100 cc.

Dissolve and filter for use.

MANIPULATION.

1. Pour off the alcohol and cover the sections with the mordant, A; allow to stand for an hour.

2. Pour off the mordant and rinse once with 75% alcohol.

3. Pour off the alcohol and cover the sections with the stain, B; and allow to stand three, six or more hours, according to nature of tissue dealt with. The thinner the section the longer it should remain in the stain.

4. Pour off the stain and rinse the sections in several changes of 92% alcohol. Reduce the colour if necessary with acidulated alcohol.

5. Clear the sections, if uncelloidinized, with oil of cajeput.

6. Transfer the sections to thin balsam and benzol, and when convenient

7. Mount the sections in balsam.

No. 7. PICRO-CARMINATE OF AMMONIA.

A

Picric acid crystals 9 grams in 400 cc. of 92% alcohol.

Best carmine 2 grams in 40 cc. of liquid ammonia.

Dissolve the two mixtures, and then add to the carmine solution, 400 cc. of water, and then add to this the picric acid solution. Filter for use.

B

A saturated solution of ammonium picrate in 92% alcohol.

Manipulation.

1. Pour off the alcohol and cover the sections with *A*, and allow to stand one hour.
2. Pour off *A*, and rinse the sections quickly with alcohol.
3. Pour off the alcohol and cover the sections with *B*, and allow to stand fifteen minutes.
4. Pour off *B*, and rinse the sections two or three times with alcohol.
5. Pour off the alcohol and cover the sections with oil of cajeput to clarify.
6. Transfer the sections to thin balsam and benzol.
7. Mount the sections in balsam.

No. 8. ANILINE PICRATE AND GOSSYPIMINE.

For Double Staining, Vegetable, and Animal Tissues.

Sections to be stained from water.

A

Aniline blue crystals	3 grams.
Picric acid crystals	2 grams.
Water	960 cc.

Dissolve the aniline blue crystals in the water, then add the picric acid; filter for use.

B

Gossypimine crystals	2 grams.
Water	900 cc.
Alcohol 92%	60 cc.

Dissolve and filter for use.

MANIPULATION.

1. Pour off the water and cover the sections with *A*, and allow to stand thirty minutes.
2. Pour off *A*, and rinse the sections in two or three changes of 25% alcohol, using a trace of acid to reduce the colour if necessary.
3. Pour off the alcohol and cover the sections with *B*, and allow to stand thirty minutes.
4. Pour off *B*, and rinse the sections quickly with 75%, finishing off with 92% alcohol.
5. Clear the sections, if uncelloidinized, in oil of cajeput.
6. Transfer the sections to thin balsam and benzol.
7. Mount the sections in balsam.

In working with aniline blue and gossypimine, it may be found advisable to reverse the order of manipulation by staining first with *B*; this will be found to be the case when the tissues dealt with are fully formed or highly lignified, *i.e.*, that stain must be used first for which the tissues have the greatest affinity, and retains it the most persistently. In the knowledge, therefore, of the *nature* of the tissues dealt with lies the secret of all good staining.

No. 9. HÆMATOXYLIN AND GOSSYPIMINE.

To double stain sections with hæmatoxylin and gossypimine, substitute hæmatoxylin for aniline picrate and manipulate as for No. 8, using acidulated alcohol to reduce the hæmatoxylin colour if necessary.

No. 10. ANILINE BLUE.

A Single Stain for Vegetable Tissue.

Aniline blue crystals ... ... 1 gram.
Alcohol 92% ... ... 300 cc.

Dissolve and filter for use.

Manipulation.

1. Pour off the alcohol and cover the sections with the stain; allow to stand thirty minutes.
2. Pour off the stain and rinse the sections in two or three changes of alcohol; using the acidulated alcohol to reduce the colour if necessary.
3. Clear the sections, if uncelloidinized, in oil of cajeput.
4. Transfer the sections to thin balsam and benzol, and when convenient
5. Mount the sections in balsam.

No. 11. MALACHITE GREEN.—A Counter Stain.

Malachite green ... ... 1 gram.
Alcohol 92% ... ... 300 cc.

Dissolve and filter for use.

Manipulation.

1. After staining with carmine, formula No. 1,
2. Pour off the alcohol and cover the sections with the malachite green stain, and allow to stand fifteen to thirty minutes.
3. Pour off the stain and rinse the sections *quickly* in two or three changes of alcohol; and as soon as the *carmine* stain stands out *clearly* pour off the alcohol and
4. Cover the sections with oil of cajeput to clarify.
5. Transfer the sections to thin balsam and benzol, and when convenient
6. Mount the sections in balsam.

No. 12. **SAFRANIN.—A Counter Stain.**

Safranin	3 grams.
Water	430 cc.
Alcohol 92%	540 cc.

Dissolve by heat, when cold filter for use.

MANIPULATION.

1. After staining with hæmatoxylin formula No. 3,
2. Pour off the alcohol and cover the sections with safranin, and allow to stand thirty minutes.
3. Pour off the stain and rinse the sections *quickly* in two or three changes of alcohol, and as soon as the hæmatoxylin colour stands out clearly, pour off the alcohol and
4. Cover the sections with oil of cajeput to clarify.
5. Transfer the sections to thin balsam and benzol, and, when convenient,
6. Mount the sections in balsam.

No. 13. **EOSIN.—A Counter Stain.**

Eosin	1 gram.
Alcohol 92%	300 cc.

Dissolve and filter for use.

Eosin may be employed as a counterstain with either aniline blue or hæmatoxylin, the manipulation being exactly the same as for Nos. 11 and 12.

MOUNTING MEDIA, etc.

CANADA BALSAM.

Canada balsam is supplied commercially in its crude condition containing essential oils and similar substances, which must be eliminated by evaporation before the balsam can be used for microscopical purposes.

Place the balsam in an evaporating pan and gently heat over a compound bunsen flame, stirring frequently, until when a drop of the hot balsam is allowed to fall into a vessel of cold water, it at once sets brittle. The balsam should now be allowed to cool, but not to set hard, when it may be thinned down to the required consistency with *pure rectified benzol*, or with *pure xylol* as required. *Filter for use.*

Gum Dammar.

Make a saturated solution of gum dammar in rectified benzol, and filter for use.

Glycerine Jelly.

Finest French gelatine	1 oz.
Water	6 ozs.
Pure glycerine, by weight	7 ozs.

Soak the gelatine in the water for two hours; then add the glycerine, warm until dissolved, and then add 1% of pure carbolic acid as a preservative, and filter for use with the aid of a hot-water jacket.

Farrant's Medium.

Arsenious acid	1 gram.
Water	200 cc.
Gum acacia	130 grams.
Pure glycerine	100 cc.

Dissolve the acid and the gum in the water by heat; then add the glycerine, and filter for use.

Glycerated Water.

Distilled water	225 cc.
Pure glycerine	75 cc.
Corrosive sublimate	1 gram.

Filter for use.

Carbolized Water.

Distilled water	300 cc.
Pure carbolic acid	1 cc.

Filter for use.

Bleaching Solution.

A

Chlorinated lime	4 ozs.
Water	10 ozs.

Stir at intervals for an hour.

B

Sodium carbonate	4 ozs.
Water	10 ozs.

Dissolve.

Pour off the clear liquid from *A*, and to this add *B*, and allow to stand for an hour to settle; filter for use.

Sections must always be placed in water before they are bleached.

Manipulation.

1. Pour off the water and cover the sections with the bleaching solution, allow to stand five to thirty minutes, or until the sections are bleached white.

2. Rinse the sections in several changes of water, or allow them to soak until quite free from the lime solution.

3. Transfer, or graduate if necessary, the sections to alcohol, ready for staining.

The washing process may be facilitated by the addition of a few drops of hydrochloric acid to the water.

Brown Cement.

A.	Best orange shellac pulverised	4 ozs.
	Alcohol 92%	300 cc.
B.	A saturated solution of pure rubber in petroleum ether ...	60 cc.
C.	Best gold size...	120 cc.

Add *B* and *C* to *A*. Filter for use.

Oxide of Zinc.

Make a saturated solution of gum dammar in pure benzol; filter this into a mortar, and add as much oxide of zinc as the solution will take up by pounding. Now thin down to the required consistency with pure benzol, and filter through fine muslin. Now add to the solution as much gold size as will impart to it a creamy tint, when shaken up.

Asphaltum.

A saturated solution in pure benzol. Filter for use.

Chapter IV.

Type preparations.

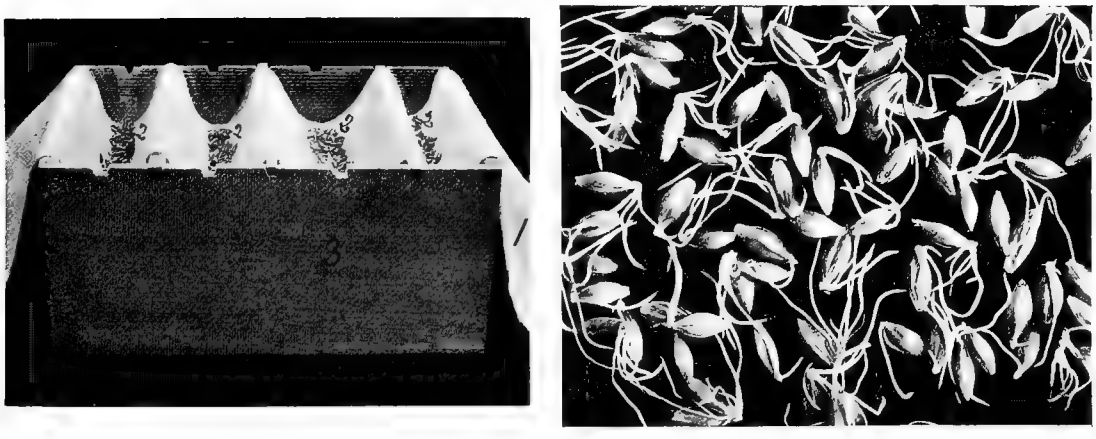
ROOT STRUCTURE.

Root of Buttercup, 'Ranunculus acris,' $^1/_{16}$ of an inch in diameter.
Fixed with 92% alcohol.
Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.
Manipulate Nos. 3 and 9.
Compare the preparation with Fig. 1. Plate I.

Root of Pine, 'Pinus sylvestris,' $^1/_{16}$ of an inch in diameter.
Fixed with 92% alcohol.
Cut transverse sections $^1/_{2000}$ of an inch in thickness. Place the sections in alcohol.
Bleach the sections as per instructions.
Manipulate Nos. 3 and 12.
Compare the preparation with Fig. 2. Plate I.

Root of Yellow Iris, 'Iris Pseudoacorus,' $\frac{1}{8}$ of an inch in diameter.
Fixed in 92% alcohol.
Cut transverse sections $^1/_{750}$ of an inch in thickness. Place the sections in alcohol.
Bleach the sections.
Manipulate Nos. 3 and 12.
Compare the preparation with Fig. 3. Plate I.

ROOT OF INDIAN CORN, 'Zea maize,' $^{3}/_{16}$ of an inch in diameter.
Fixed in 92% alcohol.
Cut transverse sections $^{1}/_{400}$ of an inch in thickness. Place the sections in alcohol.
Bleach the sections.
Manipulate Nos. 1 and 11.
Compare the preparation with Fig. 4. Plate I.



A. B.

FIG. 26.—Germinating box. A. 3, wood box, with strips of wood laid across the open top, over which a strip of flannel is let into folds. 1, loose ends of flannel leading to vessels containing water, which feeds the seed by osmosis. 2, grains of barley in the folds of the flannel. B. The seeds after being germinating for 6 days.

DEVELOPING RADICLES OF BARLEY.

Place some grains of barley in the germinating box, Fig. 26, allow them to germinate for twelve hours (the flannel must be damp when the grains are placed in). Now take out the grains, and with a sharp section knife cut them in half transversely and place the *germinal half* direct into the chromo-acetic solution, and allow them to 'fix' for twenty-four hours; now wash and graduate to alcohol as directed (see collecting and fixing of tissues).

Celloidinize the specimens.

Cut transverse sections $^{1}/_{1500}$ of an inch in thickness through the radicle end of the germ; place the sections in alcohol.

Manipulate No. 6.

Compare the preparation with Fig. 5. Plate II.

ENTIRE PLANT OF DUCKWEED, 'Lemna minor.' After fixation and washing:—

1st. Place the specimen in equal parts of alcohol, glycerine, and water; allow to stand six hours.

2nd. Place the specimens in two parts of pure glycerine and one part each of alcohol and water; allow to stand three hours.

3rd. Place the specimens in pure glycerine; allow to stand one hour.

4th. Mount the preparation in glycerine jelly.

Compare the preparation with Fig. 6. Plate II.

STEM STRUCTURE.

YOUNG STEM OF BEAN, 'Vicia faba.' Fixed with chromo-acetic solution. Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Manipulate Nos. 1 and 11.

Compare the preparation with Fig. 7. Plate III.

STEM OF BUTTERCUP, 'Ranunculus acris.' Fixed with 92% alcohol. Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Bleach the sections.

Manipulate Nos. 1 and 11.

Compare the preparation with Fig. 8. Plate III.

STEM OF WOUNDWORT, 'Stachys sylvatica.' Fixed with 92% alcohol. Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Bleach the sections.

Manipulate No. 7.

Compare the preparation with Fig. 9. Plate III.

STEM OF TAMUS, 'Tamus communis.' Fixed with 92% alcohol.
Cut transverse sections $^1/_{400}$ of an inch in thickness. Place the sections in alcohol.
Bleach the sections.
Manipulate Nos. 1 and 11.
Compare the preparation with Fig. 10. Plate III.

STEM OF MARESTAIL, 'Hippuris vulgaris.' Fixed with the chromo-acetic solution.
Cut transverse sections $^1/_{300}$ of an inch in thickness. Place the sections in alcohol.
Bleach the sections.
Manipulate Nos. 1 and 11.
Compare the preparation with Fig. 11. Plate IV.

STEM OF LIMNANTH, 'Limnanthemum nymphæoides.'
Fixed with the chromo-acetic solution.
Cut transverse sections $^1/_{300}$ of an inch in thickness. Place the sections in alcohol.
Manipulate Nos. 3 and 12.
Compare the preparation with Fig. 12. Plate IV.

STEM OF SUNFLOWER, 'Helianthus annuus.' Fixed with 92% alcohol.

1. Cut transverse sections $^1/_{300}$ of an inch in thickness. Place the sections in alcohol.
2. Cut tangential longitudinal sections $^1/_{1200}$ of an inch in thickness. Place the sections in alcohol; the line of cut must pass through the inner margin of a primary bundle.

Bleach the sections.
Manipulate Nos. 1 and 11.
Mount both the sections on one slide.
Compare the preparations with Figs. 13 and 14. Plate IV.

STEM OF LIME-TREE, 'Tilia europœa.' Fixed with 92% alcohol.

a. Cut transverse sections $^1/_{750}$ of an inch in thickness. Place the sections in alcohol.

b. Cut tangential longitudinal sections $^1/_{1200}$ of an inch in thickness, passing through the phloem. Place in alcohol.

c. Cut tangential longitudinal sections $^1/_{1000}$ of an inch in thickness, passing through the xylem. Place in alcohol.

Bleach the three series of sections.

Manipulation for *a* Nos. 1 and 11; for *b* and *c*, Nos. 3 and 12.

Mount all the sections on one slide, as in the order named.

Compare the preparation with Figs. 15, 16 and 17. Plate V.

STEM OF DRACÆNA, 'Cordyline rubra.' Fixed with 92% alcohol.

Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Bleach the sections.

Manipulate Nos. 1 and 11.

Compare the preparation with Fig. 18. Plate V.

STEM OF PINE-TREE, 'Pinus sylvestris.' Fixed with chromo-acetic solution. For development and formation of parts.

a. Cut longitudinal-median sections through apex of young stem, $^1/_{1000}$ of an inch in thickness.

b. Cut transverse sections $^1/_{750}$ of an inch in thickness, half-inch below growing point.

c. Cut transverse sections $^1/_{750}$ of an inch in thickness, two inches below growing point.

d. Cut radial longitudinal sections $^1/_{750}$ of an inch in thickness, two inches below growing point.

Place each group of sections in alcohol, as cut.

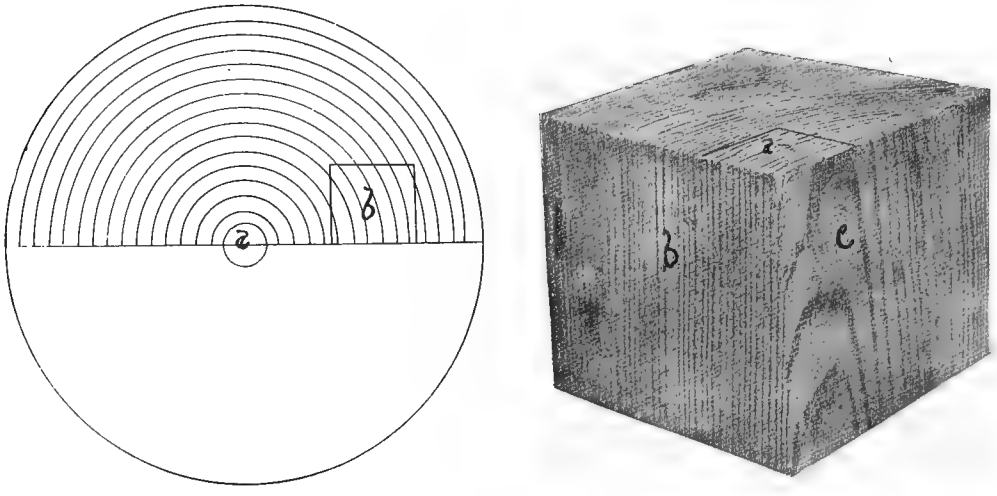
Manipulation for *a*, No. 6. Manipulation for *b*, *c*, *d*, No. 9.

Compare the preparations with Figs. 19, 20, 21, 22 Plate VI.

Also Fig. 23. Plate VII.

STRUCTURE OF TIMBER, OLD WOOD OF PINE, 'Pinus sylvestris.'

Prepare half-inch cubes of old wood of pine, Fig. 27. Steep the cubes in tepid water for twenty-four hours to soften.



A. B.

FIG. 27.—A. Diagram of a cross section of a timber tree, showing the position from which to cut out a cube for sectionizing, *a*, central axis of growth, *b*, cube. B. Cube after removal from A, *b*. *a*, transverse surface, *b*, radial-longitudinal surface, *c*, tangential longitudinal surface.

a. Cut sections $^1/_{1000}$ of an inch in thickness from the transverse surface of cube.

b. Cut sections $^1/_{1000}$ of an inch in thickness from the radial longitudinal surface of cube.

c. Cut sections $^1/_{1000}$ of an inch in thickness from the tangential longitudinal surface of cube.

Place the three series of sections in water as cut, separately.

Bleach all the sections, and transfer to 92% alcohol.

Manipulation for *a* and *c*, Nos. 3 and 12. Manipulation for *b*;——.

1. Place the sections in equal parts of alcohol, glycerine, and water; allow to stand six hours.
2. Place the sections in two parts of pure glycerine and one part each of alcohol and water; allow to stand three hours.
3. Place the sections in pure glycerine.
4. Mount the sections in glycerine jelly (without staining).

Compare the preparations with Figs. 24, 25, 26. Plate VII.

STEM OF INDIAN CORN, 'Zea mais.' Fixed with 92% alcohol.

Cut transverse sections $^1/_{300}$ of an inch in thickness. Place the sections in alcohol.

Cut radial longitudinal sections $^1/_{300}$ of an inch in thickness. Place the sections in alcohol.

Bleach the sections.

Manipulate Nos. 1 and 11.

Compare the preparation with Figs. 27 and 28. Plate VIII.

RACHIS AND RHIZOME OF FERN, 'Pteris aquilina.'
Fixed with chromo-acetic solution.

Cut transverse sections of rachis $^1/_{400}$ of an inch in thickness. Place the sections in alcohol.

Cut longitudinal sections of rhizome $^1/_{500}$ of an inch in thickness. Place the sections in alcohol.

(The line of cut for the longitudinal section must pass through both of the crescent-shaped sclerenchymatious bands, Fig. 38.

Bleach the sections.

Manipulate Nos. 1 and 11.

Compare the preparations with Figs. 29 and 30. Plate VIII.

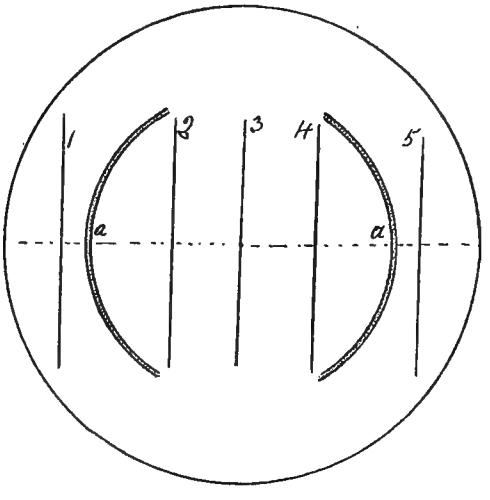


FIG. 28.—Diagram of a T.S. of rhizome of Pteris, showing the position of vessels. 1-5 position of the five scleriform vessels. *a*, the two crescent-shaped sclerenchymatious bands. The dotted line indicates the direction of cut in taking the sections.

STEM OF HORSETAIL, 'Equisetum telmateia.' Fixed with chromo-acetic solution. Cut transverse sections $^1/_{750}$ of an inch in thickness, immediately below the vegetative bud. Place the sections in alcohol.

Manipulate Nos. 1 and 11.

Compare the preparation with Fig. 31. Plate IX.

VEGETATIVE BUD OF HORSETAIL, 'Equisetum telmatia.' Fixed as above. After celloidinizing the bud,

a. Cut transverse section $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

b. Cut radial longitudinal section $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

c. Cut radial longitudinal section $^1/_{1500}$ of an inch in thickness, through an apical or lateral growing point.

Manipulation for *a*, No. 1. Manipulation for *b*, Nos. 1 and 11.

Manipulation for *c*, No. 6.

Compare the preparations with Figs. 32—34. Plate IX.

LEAF STRUCTURE.

LEAF OF PINE, 'Pinus maritimus.' Fixed with 92% alcohol. Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Bleach the sections.

Manipulate Nos. 1 and 11.

Compare the preparation with Fig. 35. Plate X.

LEAF OF MARRAM GRASS, 'Ammophila arundiacea.' Fixed with 92% alcohol. Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Bleach the sections.

Manipulate Nos. 1 and 11.

Compare the preparation with Fig. 36. Plate X.

LEAF OF ORCHIS, 'Cypripedium Sp.' Fixed with 92% alcohol.
Cut transverse sections $^1/_{400}$ of an inch in thickness. Place the sections in alcohol.
Bleach the sections.
Manipulate Nos. 1 and 11.
Compare the preparation with Fig. 37. Plate XI.

LEAF OF CYCAS, 'Cycas revoluta.' Fixed with the chromo-acetic solution.
Cut transverse sections $^1/_{750}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 8.
Compare the preparation with Fig. 38. Plate XI.

LEAF OF BOTTLE-BRUSH, 'Metrosideros verni.'
Fixed with the chromo-acetic solution.
Cut transverse sections $^1/_{2688}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 8.
Compare the preparation with Figs. 39—40. Plate XI.

LEAF OF TRADESCANTIA, 'Tradescantia discolor.'
Fixed with the chromo-acetic solution.
Cut transverse sections $^1/_{500}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 8.
Compare the preparation with Fig. 41. Plate XII.

CUTICLE FROM LEAF OF MONKEY-PUZZLE, 'Araucaria imbricata.'
Preserved in 92% alcohol.

a. Cut the leaves into half-inch squares with a sharp section-knife.
b. Place the pieces in a test-tube with a mixture of equal parts of nitric acid and water.

c. Boil over the bunsen flame for two or three minutes.

d. Pour the contents of the test-tube into cold water, shake well for two or three minutes.

e. Pick out the separated cuticles and place them in clean water to wash for an hour or two.

If the intermediate tissue has not been completely removed, the boiling process must be repeated, at the same time great care must be taken not to carry the boiling beyond this point, or the guard cells of the stomata will also be removed. After washing the cuticles

f. Transfer to 92% alcohol.

Manipulate No. 6.

Compare the preparation with Fig. 42. Plate XII.

STOMATA IN GREEN STEM, SHEE OAK, 'Casuarina equisetifolia.' Developing twig $^1/_{16}$in. diameter. . Fixed with chromo-acetic solution.

Cut transverse sections $^1/_{1200}$ of an inch in thickness. Place the sections in alcohol.

Manipulate Nos. 1 and 11.

Compare the preparation with Fig. 43. Plate XII.

STING OF NETTLE, 'Urtica dioica.' Fixed with the chromo-acetic solution.

With a sharp scalpal remove longitudinal strips of cuticle, with its stings, from the midrib of the leaf, divide the strips as required and place in alcohol.

Manipulate Nos. 1 and 11.

Compare the preparation with Fig. 44. Plate XII.

PHYLLODIUM OF ACACIA, 'Acacia decurrens.' Fixed with chromo-acetic solution.

Cut transverse sections $^1/_{2000}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 8.

Compare the preparation with Fig. 45. Plate XIII.

CLADODE OF BUTCHER'S BROOM, 'Ruscus aculeatus.'

Fixed with chromo-acetic solution.

Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 8.

Compare the preparation with Fig. 46. Plate XIII.

LEAF-BUD OF BEECH, 'Fagus sylvatica.' Fixed with the chromo-acetic solution.

Celloidinize the specimen.

Cut transverse sections $^1/_{750}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 1.

Compare the preparation with Fig. 47. Plate XIII.

LEAF-BUD OF ASH TREE, 'Fraxinus excelsior.'

Fixed with chromo-acetic solution.

Celloidinize the specimen.

Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 1.

Compare the preparation with Fig. 48. Plate XIII.

LEAF-BUD OF SYCAMORE, 'Acer Pseudo-platanus.'

Fixed with chromo-acetic solution.

Celloidinize the specimen.

Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 1.

Compare the preparation with Fig. 49. Plate XIV.

DEFOLIATION IN SYCAMORE, 'Acer Pseudo-platanus.' Fixed with 92% alcohol.
Specimens collected about the middle of September.

Cut longitudinal median sections $^1/_{600}$ of an inch in thickness, of a lateral branch, passing through the opposite leaf-stalks of the current year, and the developing leaf-buds of next year. Place the sections in alcohol.

Bleach the sections.

Manipulate Nos. 1 and 11.

Compare the preparation with Figs. 50 and 51. Plate XIV.

FLORAL STRUCTURE.

FLOWER-BUD OF POPPY, 'Papaver rhæas.' Fixed with 92% alcohol.

Celloidinize the specimen.

Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 1.

Compare the preparation with Fig. 52. Plate XV.

FLOWER-BUD OF WALLFLOWER, 'Cheiranthus Cheiri.' Fixed with 92% alcohol.

Celloidinize the specimen.

Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 1.

Compare the preparation with Fig. 53. Plate XV.

FLOWER-BUD OF DANDELION, 'Taraxacum officinalis.'
Fixed with chromo-acetic solution.

Celloidinize the specimen.

Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 1.

Compare the preparation with Fig. 54. Plate XV.

FLOWER-BUD OF PURPLE IRIS, 'Iris Germanica.' Fixed with 92% alcohol.
Celloidinize the specimen.
Cut transverse sections $^1/_{500}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 1.
Compare the preparation with Fig. 55. Plate XV.

MATURE FLOWER-BUD OF LILY, 'Lilium croceum.'
Fixed with chromo-acetic solution.
Celloidinize the specimen.
Cut transverse sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 1.
Compare the preparation with Fig. 56. Plate XVI.

IMMATURE FLOWER-BUD OF LILY, 'Lilium croceum.'
Fixed with chromo-acetic solution.
Dissect out the anthers and the ovary.
Celloidinize the specimens.

a. Cut transverse sections $^1/_{1000}$ of an inch in thickness of the anther. Place the sections in alcohol.

b. Cut transverse sections $^1/_{1000}$ of an inch in thickness of the ovary. Place the sections in alcohol.

Manipulate No. 6.
Compare the preparations with Figs. 57—58. Plate XVI.

CATKIN OF SALLOW, 'Salix capræa.' Fixed with chromo-acetic solution.
Celloidinize the specimen.
Cut longitudinal median sections $^1/_{400}$ of an inch in thickness.

Place the sections in alcohol.
Manipulate No. 8.
Compare the preparation with Fig. 59. Plate XVI.

Entire Flower of the Wheat, 'Triticum vulgaris.'
Fixed with the chromo-acetic solution.
Specimen collected before emerging from the sheath, and dissected out.
Manipulate No. 1.
Compare the preparation with Fig. 60. Plate XVII.

Stigma of Wheat, 'Triticum vulgaris.' Fixed with chromo-acetic solution.
Specimen dissected out of its sheath about ten days prior to its natural time of opening.
Manipulate No. 1.

a. Place the stigma in equal parts of alcohol, glycerine, and water.
b. Place the stigma in two parts pure glycerine, and one part each of alcohol and water.
c. Place the stigma in pure glycerine.
Allow to stand in each solution of *a* and *b* for an hour.
d. Mount the specimen in glycerine jelly.
Compare the preparation with Figs. 61—62. Plate XVII.

CELL-STRUCTURE AND CELL-CONTENTS.

Place some grains of wheat, 'Triticum vulgaris,' in the germinating box, and allow them to germinate for sixteen hours. Fix with the chromo-acetic solution, and then graduate to 92% alcohol. (See Fig. 26).
Cut longitudinal-median sections of entire seed, $^1/_{600}$ of an inch in thickness.
Place the sections in alcohol.
Manipulate No. 1.
Compare the preparation with Fig. 63. Plate XVIII.

EMBRYO OF WHEAT after germinating twenty-four hours.

Fix with the chromo-acetic solution, then graduate to 92% alcohol.

Cut longitudinal median section of embryo $^1/_{1000}$ of an inch in thickness.

Place the sections in alcohol.

Manipulate No. 6.

Compare the preparation with Fig. 64. Plate XVIII.

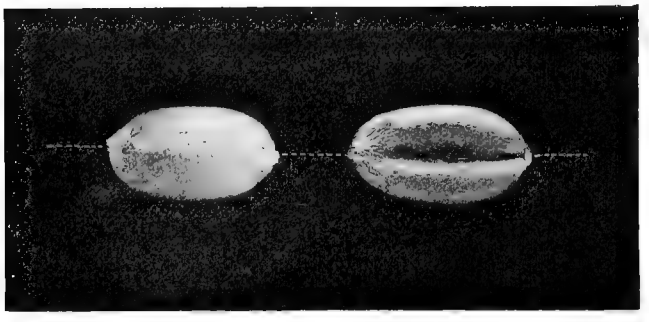


FIG. 29.—Ventral and dorsal view of a grain of wheat, the dotted line indicates the median area from which sections must be taken.

SEED OF WHEAT, germinated for twelve hours, fixed and graduated as above.

Cut transverse sections through endosperm $^1/_{1000}$ of an inch in thickness.

Place the sections in alcohol.

Manipulate No. 6.

Compare the preparation with Fig. 65. Plate XVIII.

EMBRYO OF WHEAT, after twenty-four hours germination.

Fixed with corrosive-picro-formaldehyde solution, then graduated to 92% alcohol.

Cut longitudinal median sections of embryo $^1/_{1000}$ of an inch in thickness.

Place the sections in alcohol.

Manipulate No. 6.

Compare the preparation with Fig. 66—67. Plate XIX.

EMBRYO OF WHEAT, collected before fertalization.

Fixed with chromo-acetic solution.

Cut longitudinal sections $^1/_{750}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 6.

Compare the preparation with Fig. 68. Plate XIX.

EMBRYO OF WHEAT, collected after fertilization.

Fixed with chromo-acetic solution.

Cut longitudinal sections $^1/_{750}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 6.

Compare the preparation with Fig. 69. Plate XIX.

WHEAT-RUST, 'Puccinia graminis.' Uredospores, on leaf of thistle.

Fixed with 92% alcohol.

Celloidinize the specimen.

Cut transverse sections of leaf $^1/_{1000}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 10.

Compare the preparation with Fig. 70. Plate XX.

WHEAT-RUST, 'Puccinia graminis.' Teleutospores on stem of wheat.

Fixed with 92% alcohol.

Celloidinize the specimen.

Cut transverse sections of the stem $^1/_{1500}$ of an inch in thickness. Place the sections in alcohol.

Mount without staining.

Compare the preparation with Fig. 71. Plate XX.

WHEAT-RUST, 'Puccinia graminis.' Æcidiaspores on leaf of barbery.
Fixed with 92% alcohol.
Celloidinize the specimen.
Cut transverse sections of leaf $^1/_{1500}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 10.
Compare the preparation with Fig. 72. Plate XX.

Entire plant of 'Peziza convexula.' Fixed with 92% alcohol.
Celloidinize the specimen.
Cut vertical sections of plant $^1/_{750}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 8.
Compare the preparation with Fig. 73. Plate XX.

SEA-WEED. Conceptacles of 'Fucus vesiculosus.'
Fixed with chromo-acetic solution in sea water.
Celloidinize the specimens.
Cut transverse sections of male conceptacle, $^1/_{1000}$ of an inch in thickness. Place the sections in alcohol.
Cut transverse sections of female conceptacle, $^1/_{1000}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 8.
Compare the preparations with Fig. 74—75. Plate XXI.

FERN, entire thallus of 'Gymnogramma sp.' Fixed with chromo-acetic solution.
Manipulate No. 1.
Compare the preparation with Fig. 76. Plate XXI.

FERN, thallus of 'Gymnogramma sp.' Fixed with chromo-acetic solution.
Infiltrate the specimen with paraffin.
Cut vertical sections $^1/_{5000}$ of an inch in thickness, with the mechanical microtome.
Mount the preparation as described for the paraffin method.
Manipulate No. 6.
Compare the preparation with Fig. 77. Plate XXI.

STARCH in stem of tradescantia, 'Tradescantia discolor.'
Fixed with chromo-acetic solution.
Cut transverse sections of stem $^1/_{400}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 3 and Gossypimine (8B).
Compare the preparation with Fig. 78. Plate XXII.

STARCH in stem of pellionia, 'Pellionia Daveauna.'
Fixed with chromo-acetic solution.
Cut transverse sections of stem $^1/_{400}$ of an inch in thickness. Place the sections in alcohol.
Manipulate No. 3 and Gossypimine (8B).
Compare the preparation with Fig. 79. Plate XXII.

APICAL-CELL in root of fern, 'Aspidium Filix-mas.'
Fixed with chromo-acetic solution.
Apex of developing roots collected about the middle of May.
Infiltrate the specimens with paraffin.
Cut longitudinal median sections $^1/_{6000}$ of an inch in thickness.
Mount the sections as described for the paraffin method.
Manipulate No. 6.
Compare the preparation with Fig. 80. Plate XXII.

RAPHIDES in aerial root of monstera, 'Monstera deliciosa.'

Fixed with chromo-acetic solution.

Infiltrate the spemen with paraffin.

Cut longitudinal median sections of apex of root $^1/_{3000}$ of an inch in thickness.

Mount the sections as directed for the paraffin method.

Manipulate No. 6.

Compare the preparation with Fig. 81. Plate XXII.

ENDOSPERM in seed of castor-oil plant, 'Ricinus communis.'

Fixed with 92% alcohol.

Remove the kernel from the husk, and divide in half transversely with a sharp section-knife.

Celloidinize the specimens.

Cut transverse sections $^1/_{1200}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 6.

Compare the preparation with Fig 82, (unstained). Plate XXIII.

LATICIFEROUS VESSELS in root of 'Scorzonera hispanica.'

Fixed with chromo-osmo-acetic solution.

Cut longitudinal sections $^1/_{600}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 3.

Compare the preparation with Fig. 83. Plate XXIII.

LATICIFEROUS VESSEL in stem of "Euphorbia splendens.'

Fixed with absolute alcohol.

Cut longitudinal sections of stem $^1/_{1000}$ of an inch in thickness. Place the sections in alcohol.

Manipulate No. 1 or 6.

Compare the preparation with Fig. 84. Plate XXIII.

KARYOKINESIS in root of onion, 'Allium cepa.'

Fixed with chromo-acetic solution.

Infiltrate the specimen with paraffin.

Cut longitudinal sections $^1/_{6000}$ of an inch in thickness.

Mount the sections as directed for the paraffin method.

Manipulate No. 6.

Compare the preparation with Fig. 85. Plate XXIII.

PLATE I.

ROOT STRUCTURE.

FIG. 1. Transverse section. Root of Buttercup. 'Ranunculus acris' ×75 dia. Fixed in 92% Alcohol. 1. Ground tissue containing starch. 2. Endodermis. 3. The four phloem groups. 4. The four xylem groups. Stained Hæmatoxylin and Gossypimine.

FIG. 2. Transverse section. Root of Pine. 'Pinus sylvestris' ×75 dia. Fixed in 92% Alcohol. Stained Hæmatoxylin and Safranin. 1. Cortical tissue. 2. Phloem. 3. Cambium. 4. Secondary wood. 5. Primary wood. 6. Resin duct.

FIG. 3. Transverse section. Root of Iris. 'Iris pseudo acorus' ×50 dia. Fixed in 92% Alcohol. Stained Hæmatoxylin and Safranin. 1. Primary ground tissue. 2. Endodermis. 3. Large pitted vessels. 4. Phloem elements, stained purple. 5. Xylem elements, stained red. 6. Developing lateral rootlets. 7. Central axis of root.

FIG. 4. Transverse section. Root of 'Zea mais" ×35 dia. Fixed in 92% Alcohol. Stained Borax Carmine and Malachite Green. 1. Cortical tissue. 2. Endodermis. 3. Xylem elements, stained green; phloem elements, stained pink. 4. Pith. 5. Developing lateral rootlets in various stages.

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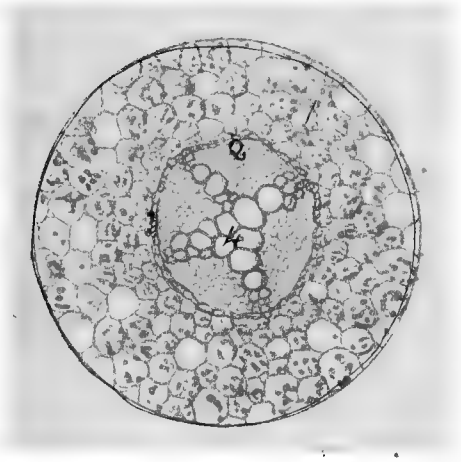

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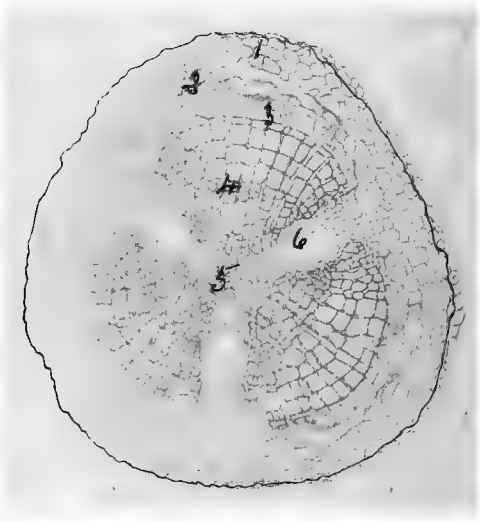

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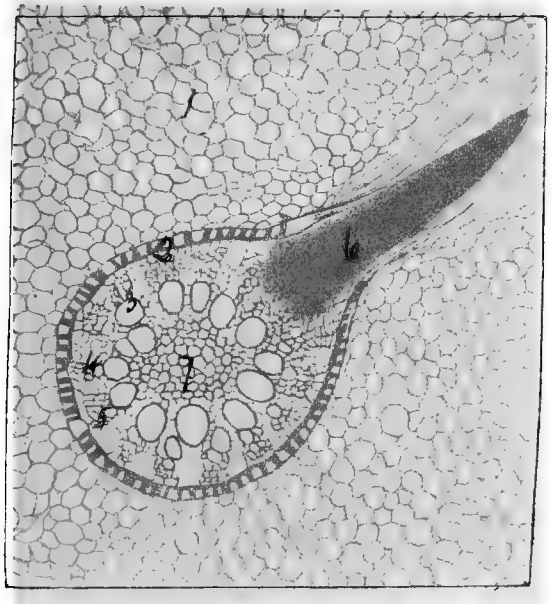

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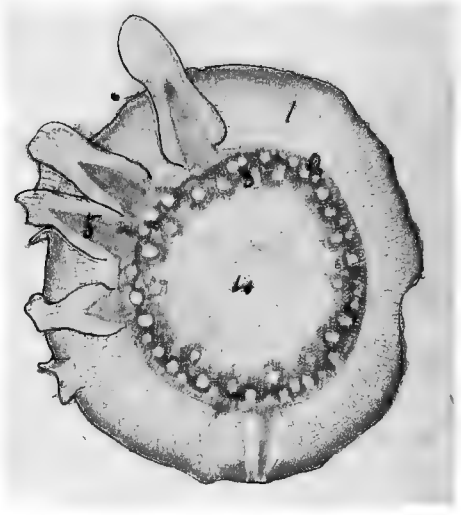

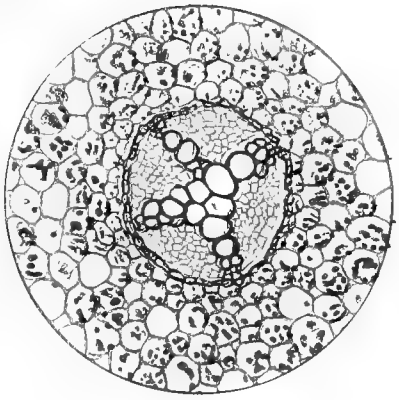

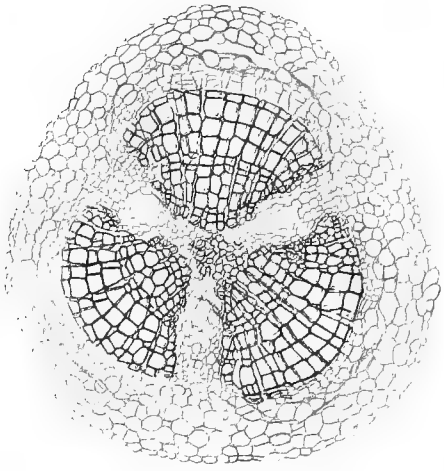

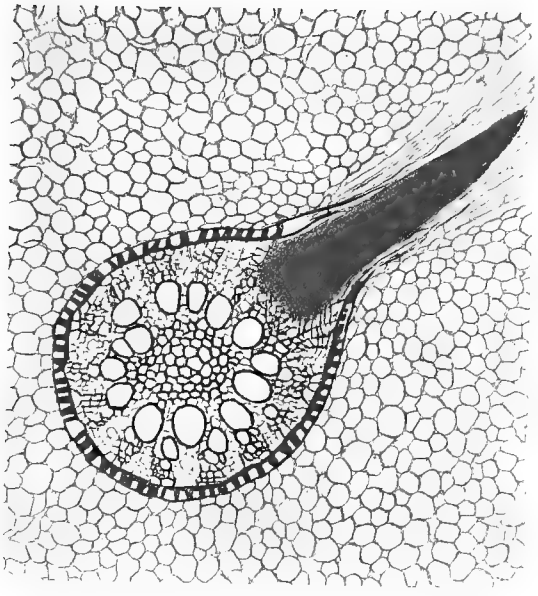

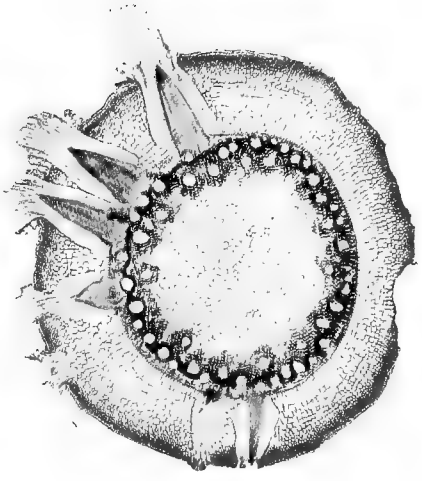

PLATE II.

ROOT STRUCTURE.

FIG. 5. Transverse section through radical end of a grain of barley, after twelve hours' germination. Fixed with Chromo-Acetic solution. Cut $^{1}/_{1500}$ in. Stained with Brazilin. 1. Aleurone layer. 2. Endosperm, containing starch. 3. The pericarp. 4. Primary nucleated tissue of scutellum. 5. Cells to form epidermis (dermatogen). 6. Cells to form cortical tissue (periblem). 7. Cells to form vascular system (plerome). 8. Central vessel of root. 9. Absorptive layer of embryo. A. The four lateral roots. B. The primary or tap-root.

FIG. 6. Entire plant of 'Lemna minor,' common Duckweed × 12 dia. Fixed with Chromo-Acetic solution. 1. The fronds containing bundles of raphides (shown as dark spots). 2. Root. 3. Root-cap. 4. Developing root.

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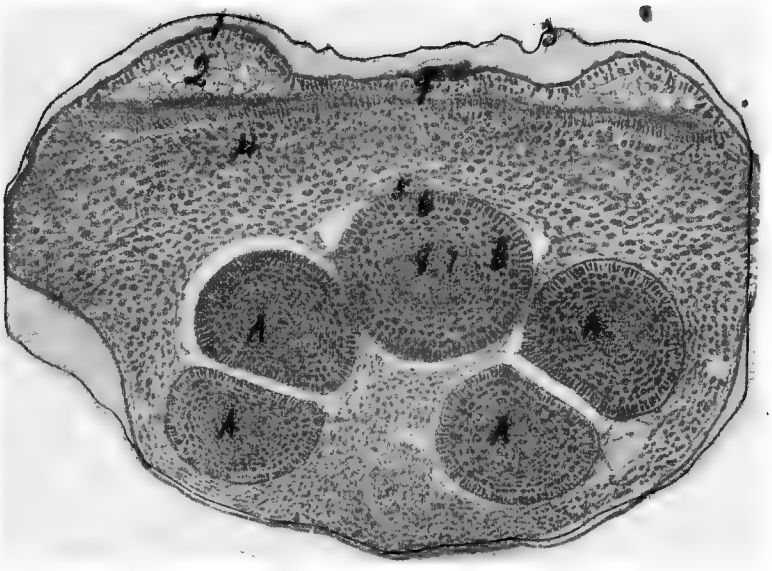

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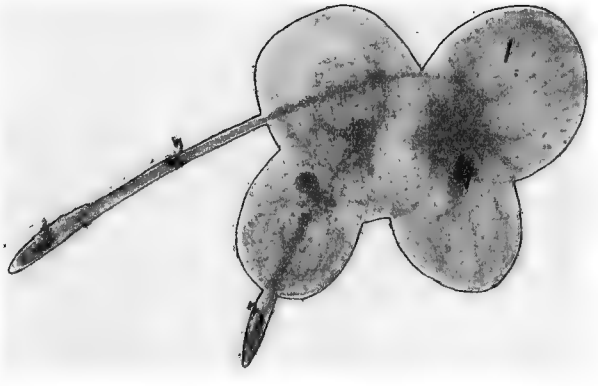

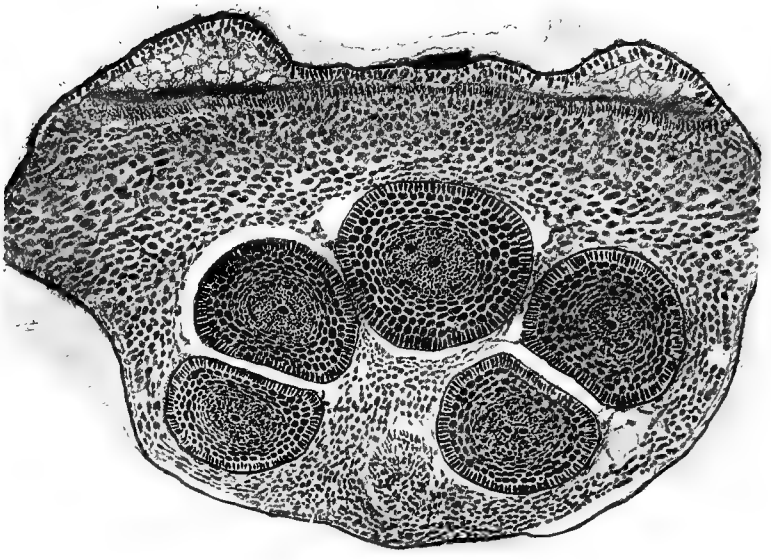

PLATE III.

STEM STRUCTURE.

FIG. 7. Transverse section through young Stem of Bean. 'Vicia faba' × 35 dia. Stained with Borax Carmine and Malachite Green. Fixed in the Chromo-Acetic solution. 1. Primary ground tissue. 2. Cambiform tissue. 3. Phloem. 4. Xylem elements. 5. Pith. 6. Traces to first leaf. The line of cut being immediately below the first leaves.

FIG. 8. Transverse section. Stem of Buttercup. 'Ranunculus acris' × 18 dia. Fixed in 92% Alcohol. Stained with Borax Carmine and Malachite Green. 1. Cortical tissue. 2. phloem elements. 3. Xylem elements. 4. Central hollow cavity of stem. 5. Primary ground tissue. 6. Primary vascular bundles. 7. Leaf trace bundle. 8. Cambium.

FIG. 9. Transverse section. Stem of Wound-wort. 'Stachys sylvatica' × 20 dia. Fixed in 92% Alcohol. Stained with Picro-carminate of Ammonia. 1. Cortical tissue. 2. Cambium. 3. Xylem elements. 4. Primary xylem bundles. 5. Primary ground tissue.

FIG. 10. Transverse section. Stem of Tamus. 'Tamus communis' × 25 dia. Fixed in 92% alcohol. Stained with Borax Carmine and Malachite Green. 1. Cortical tissue. 2. Sclerenchymatous ring. 3. Xylem elements. 4. Primary ground tissue. 5. Central cavity of stem.

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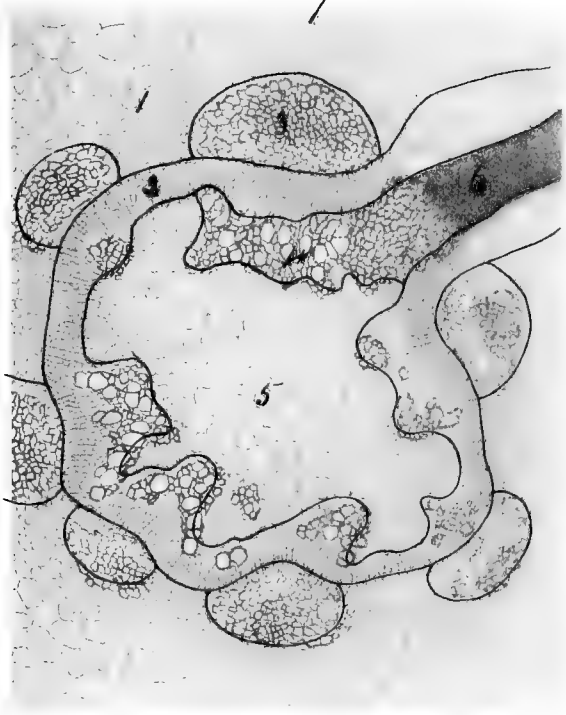

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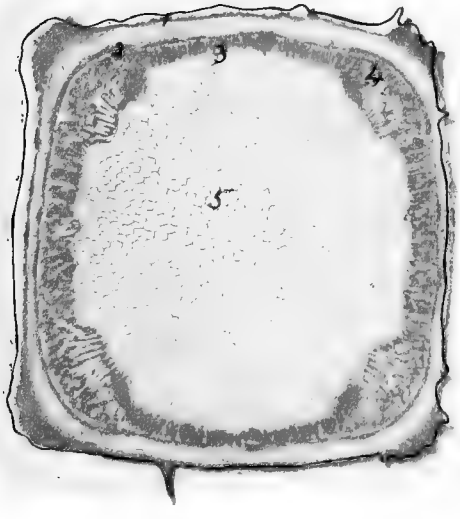

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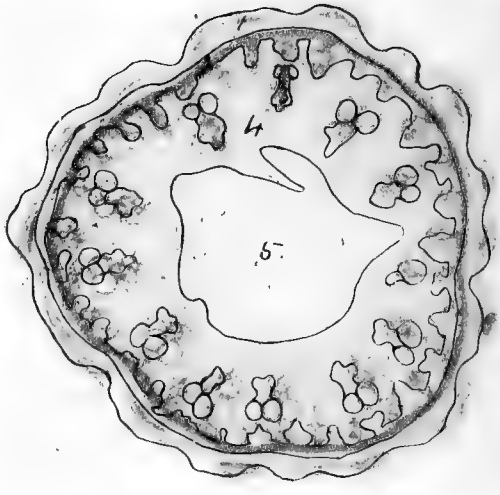

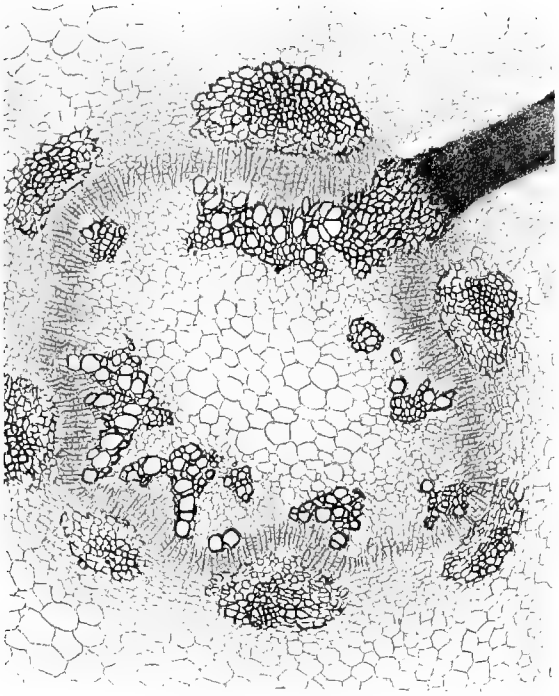

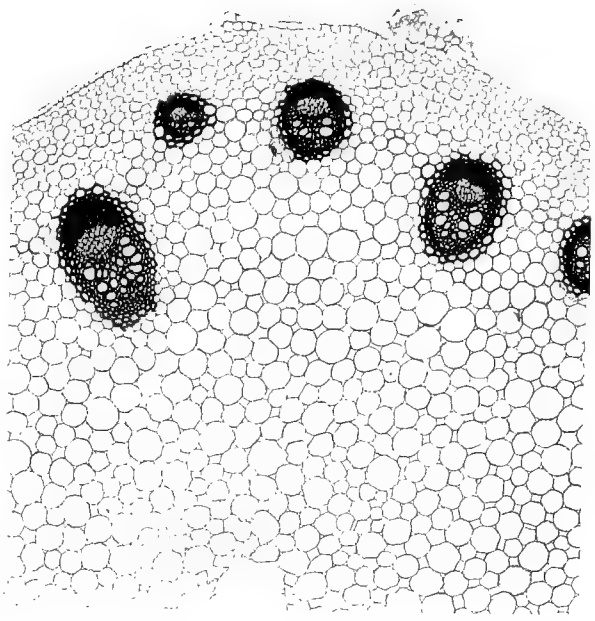

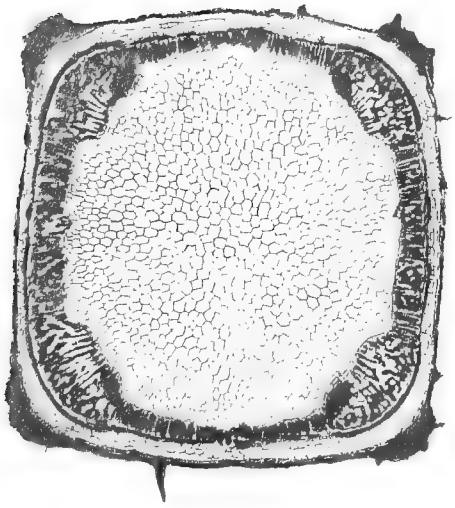

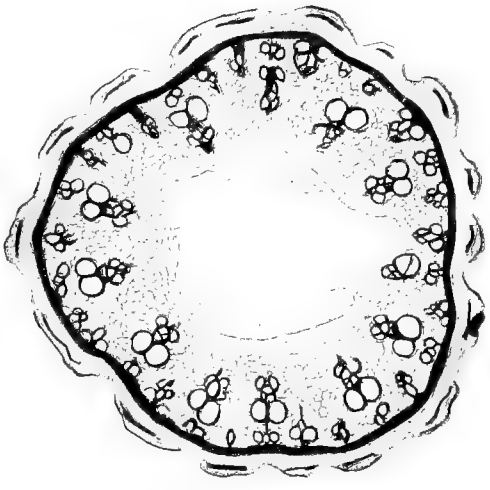

PLATE IV.

STEM STRUCTURE.

FIG. 11. Transverse section. Stem of Marestail. 'Hippuris vulgaris' × 15 dia. Fixed in Chromo-Acetic solution. Stained Borax Carmine and Malachite Green. 1. Cortical tissue. 2. Central axis of stem. 3. Xylem elements. 4. Cambiform tissue. 5. Phloem elements.

FIG. 12. Transverse section. Stem of Limnanth 'Limnanthemum nymphæoides' × 20 dia. Fixed in Chromo-Acetic solution. Stained Hæmatoxylin and Safranin. 1. Cortical tissue. 2. Idioblasts. 3. Intercellular space. 4. Xylem elements. 5. Central axis of stem. 6. Primary ground tissue formed of radialy elongated cells.

FIG. 13. Transverse section of Sunflower. 'Helianthus annuus' × 20 dia. Fixed in 92% Alcohol. Stained Borax Carmine and Malachite Green. 1. Cortical tissue. 2. Primary ground tissue. 3. Cambium ring. 4. Xylem elements. 5. Phloem elements.

FIG. 14. Tangential longitudinal section. Stem of Sunflower. 'Helianthus annuus' × 65 dia. Cut $^1/_{1200}$ in. The line of cut passing through the inner margin of a primary bundle. 1. Primary ground tissue. 2. Fibro-vascular bundle. 3. Spiral vessel.

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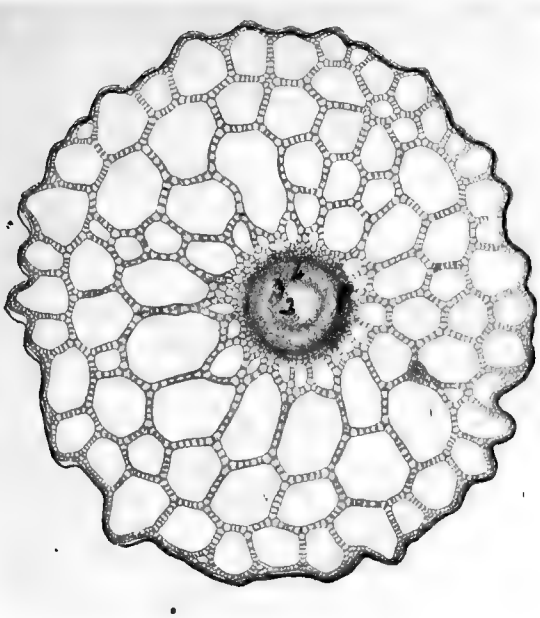

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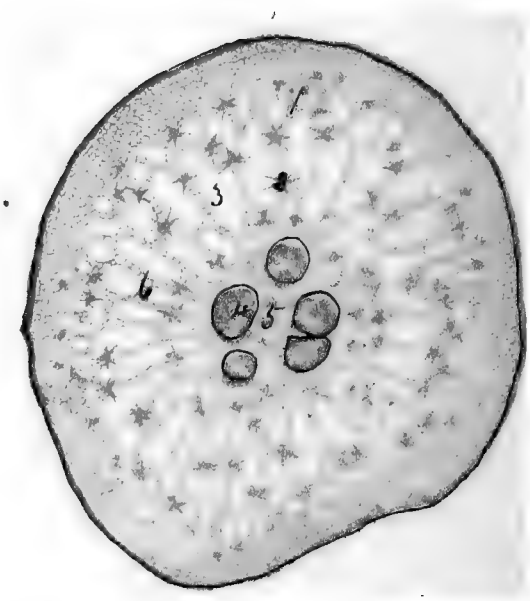

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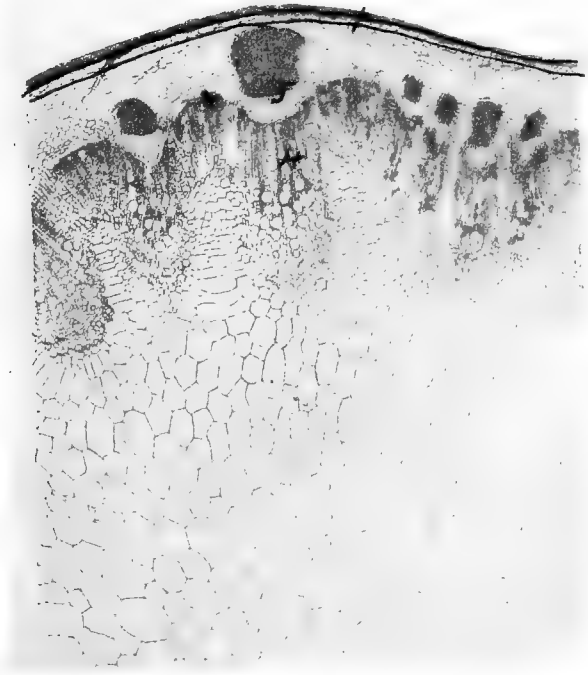

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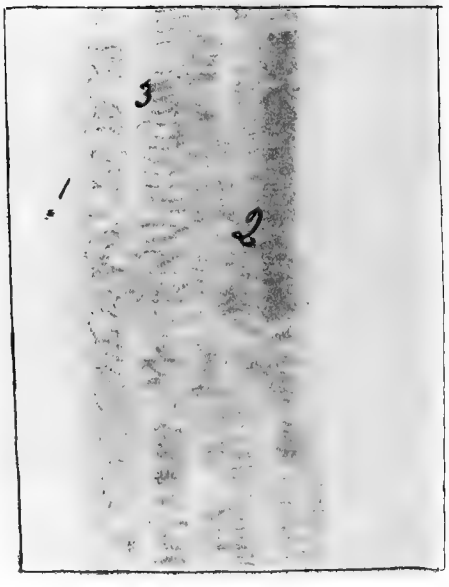

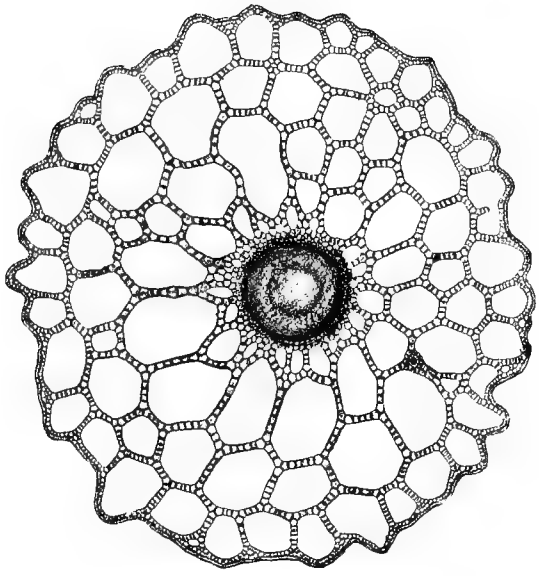

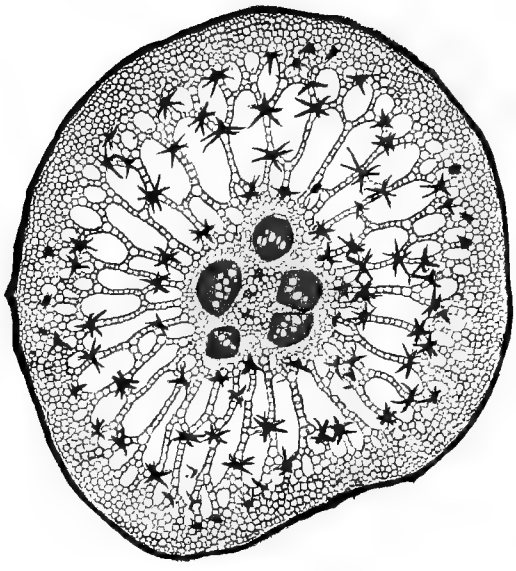

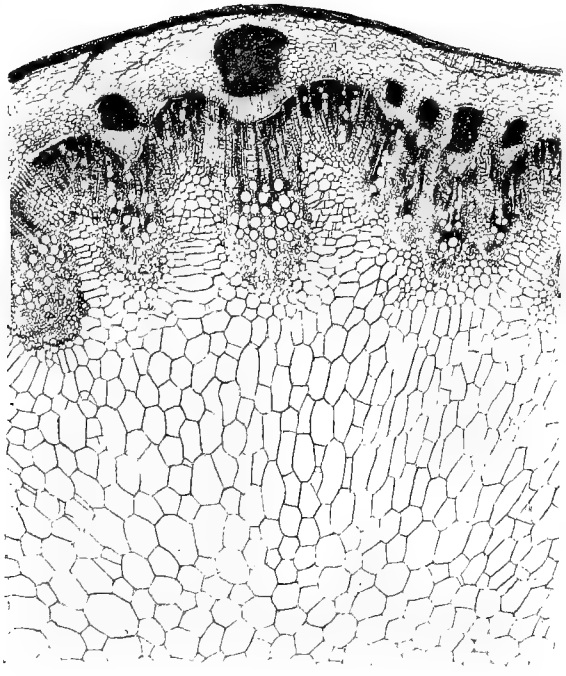

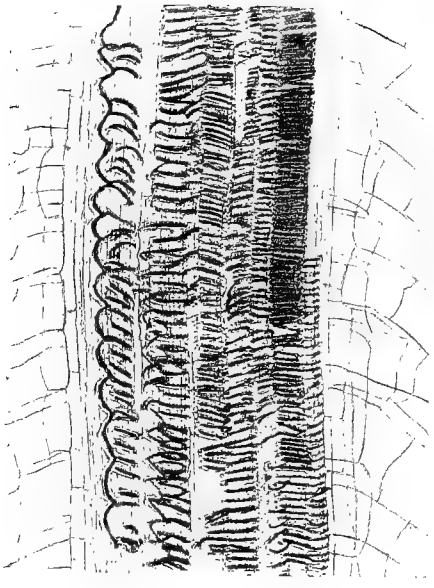

PLATE V.

STEM STRUCTURE.

FIG. 15. Transverse section. Stem of Lime Tree, 'Tilia europœa' ×40 dia. Fixed in 92% Alcohol. Stained with Borax Carmine and Malachite Green. 1. Cortical tissue. 2. Primary medullary ray. 3. bast (phloem). 4. Cambium-ring. 5. Xylem elements, of about three years' growth. 6. Central axis of pith. 7. Medullary sheath containing protoxylem elements.

FIG. 16. Tangential longitudinal section. Stem of Lime Tree. 'Tilia europœa' ×25 dia. The line of cut passing through the 'bast area.' Cut $^{1}/_{1200}$ in. Stained Hæmatoxylin and Safranin. 1. Ground tissue (medullary ray). 2. Bast fibres.

FIG. 17. Tangential longitudinal section. Stem of Lime Tree. 'Tilia europœa' ×50 dia. The line of cut passing through xylem elements. Stained with Hæmatoxylin and Safranin. 1. Connective tissue. 2. Pitted vessels. 3. Medullary ray.

FIG. 18. Transverse section. Stem of Dracæna. 'Cordyline rubra' ×50 dia. Fixed in 92% Alcohol. Stained Borax Carmine and Malachite Green. 1. Cortical tissue. 2. Cambiform zone in which new bundles are being developed. 3. Secondary vascular bundles in various stages of development. 4. Primary vascular bundles.

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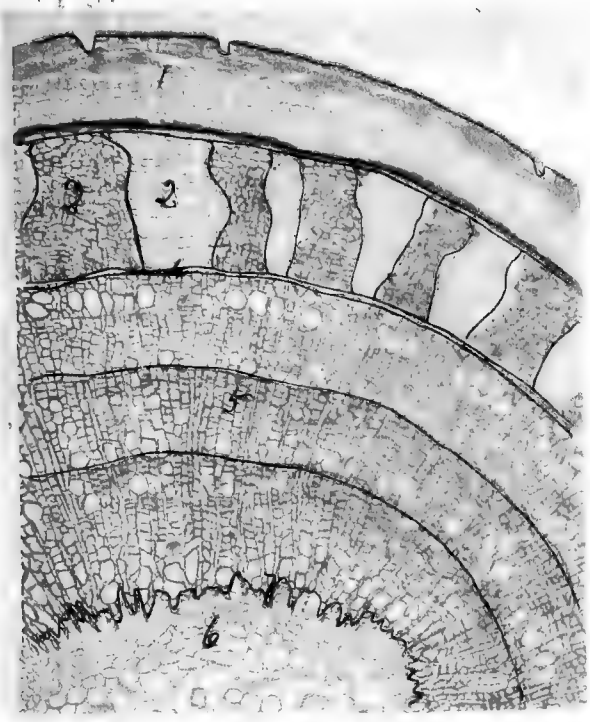

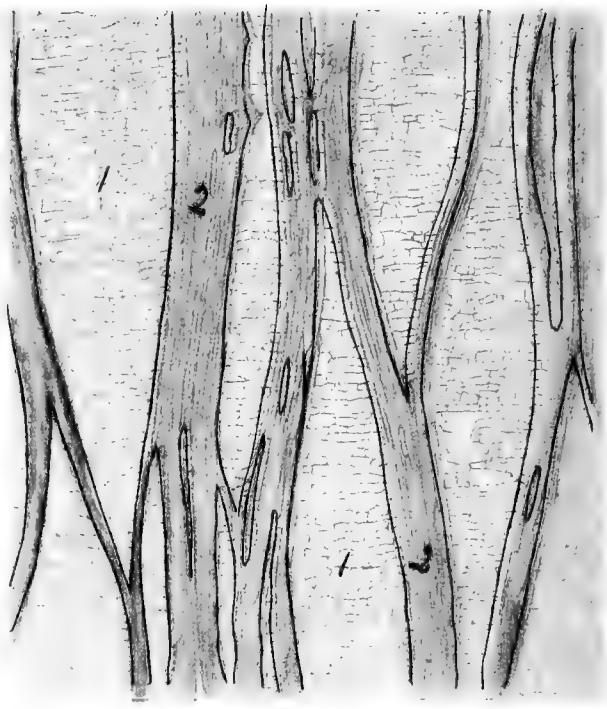

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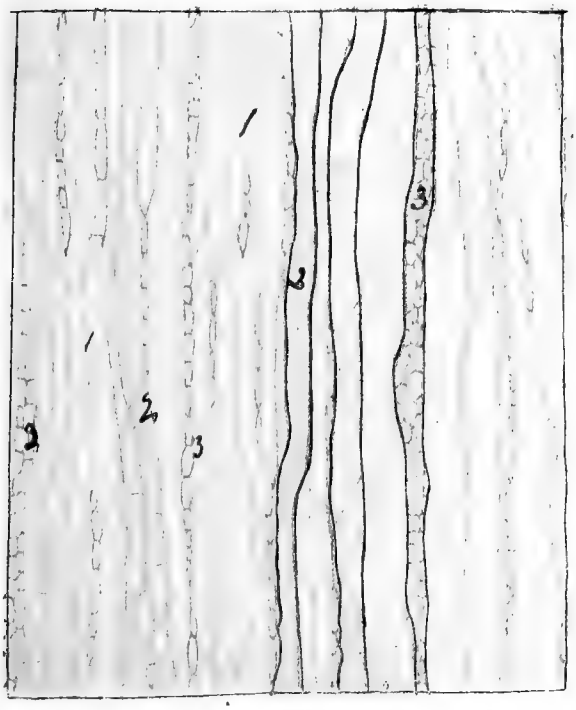

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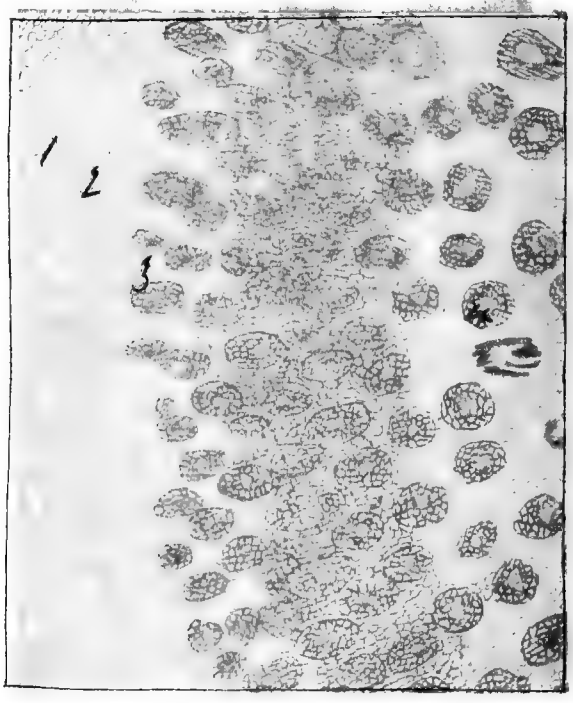
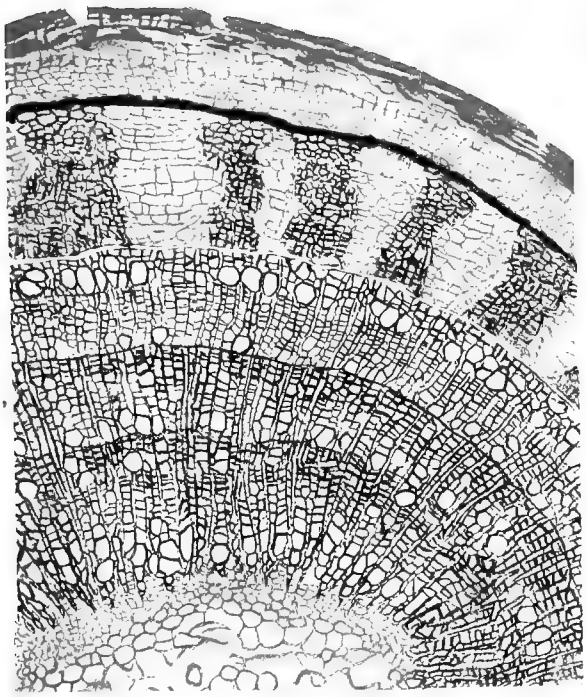
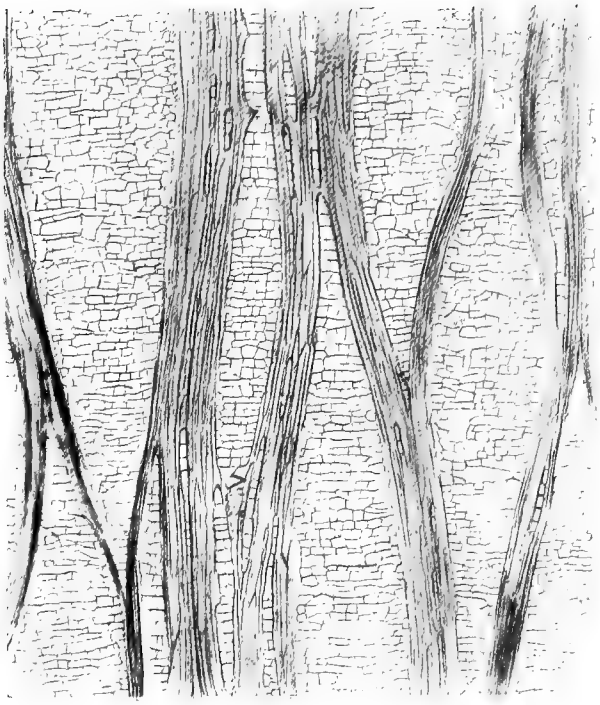
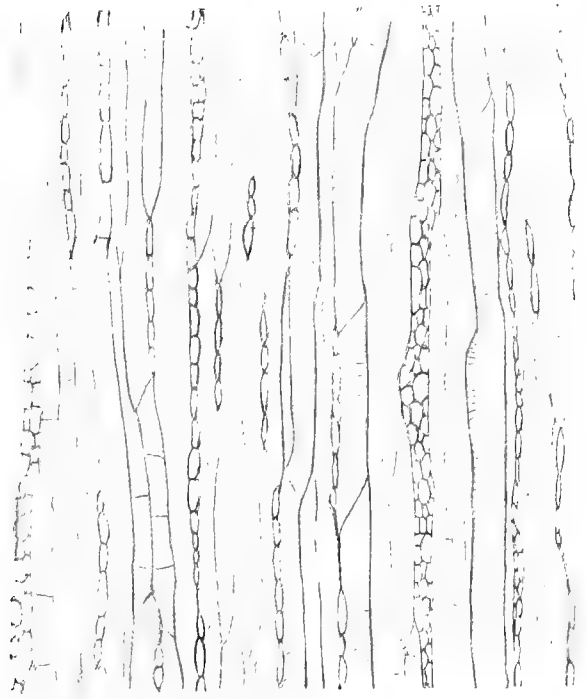








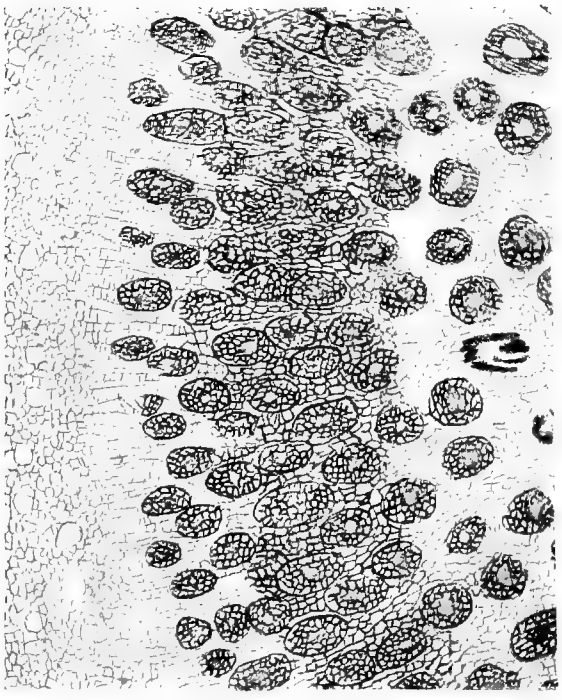


PLATE VI.

STEM STRUCTURE.

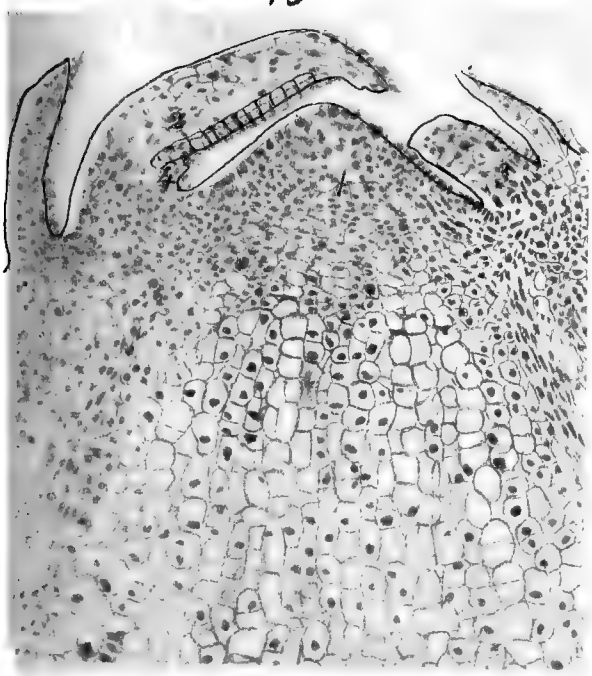
FIG. 19. Longitudinal median section of apex of Stem of Pine. 'Pinus sylvestris.' Fixed with the Chromo-Osmic-Acetic solution × 75 dia. Stained with Brazilin. 1. Zone of apical tissue in active state of division. 2 and 3, axillary buds in various stages of development. 4, primary nucleated tissue.

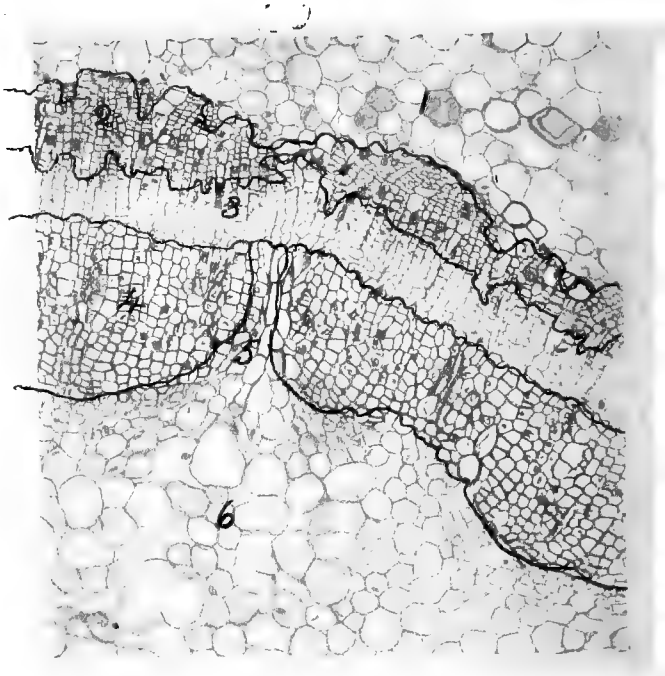
FIG. 20. Transverse section, half-inch below growing point. Stem of Pine × 75 dia. Stained with Aniline-picrate and Gossypimine. Tissue fixed as above. 1. Cortical tissue formed of loose cells. 2. Bast. 3. Cambiform tissue. 4, Xylem elements before the beginning of secondary thickening. 5. Primary medullary ray. 6. Primary ground tissue.

FIG. 21. Transverse section, two inches below growing point. Stem of Pine × 35 dia. Tissue fixed and stained as in Fig. 20. 1. Cortical tissue. 2, Phloem elements. 3. Cambiform tissue. 4. Xylem elements, after the commencement of secondary thickening. 5. Resin passage. 6. Central ground tissue. 7. Developing branch.

FIG. 22. Radial longitudinal section. Stem of Pine × 35 dia. Area as in Fig. 21. 1. Cortical tissue. 2. Phloem elements. 3. Xylem elements. 4. Primary central tissue. 5. Cambium. 6. Developing branch.

19





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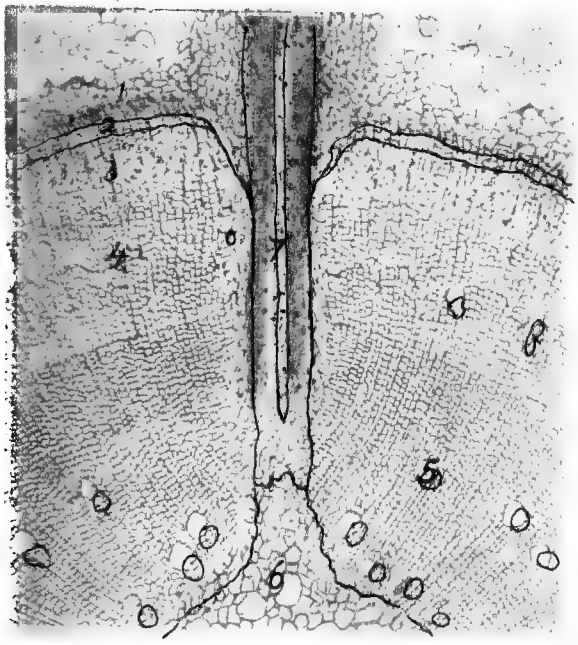

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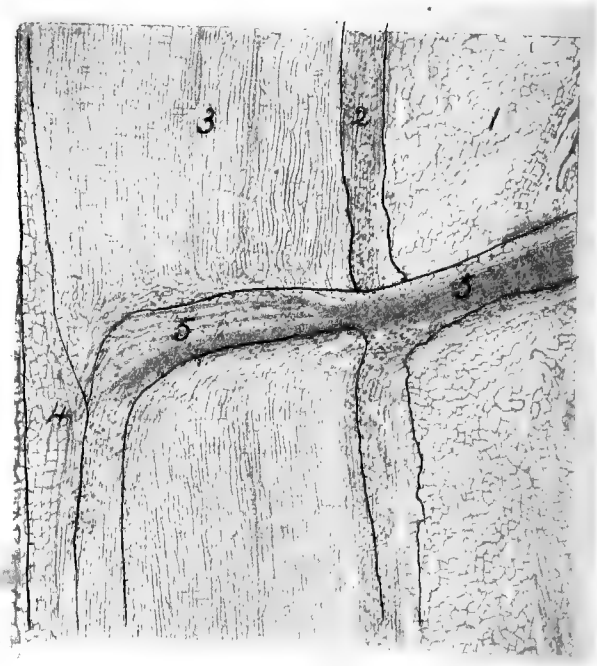
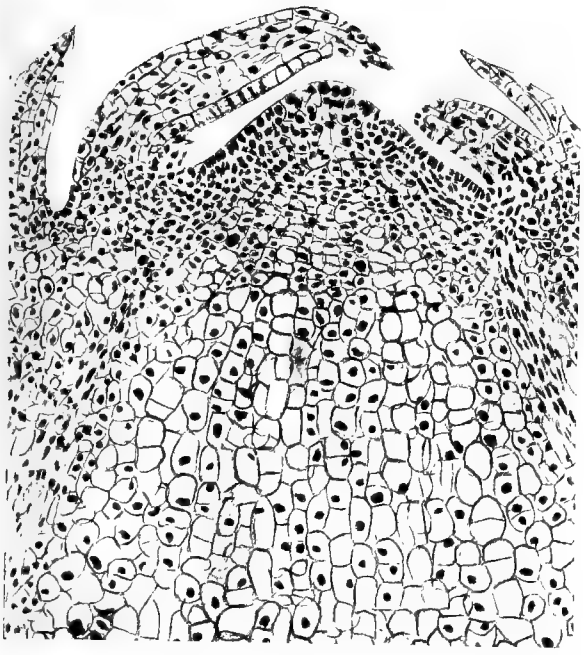
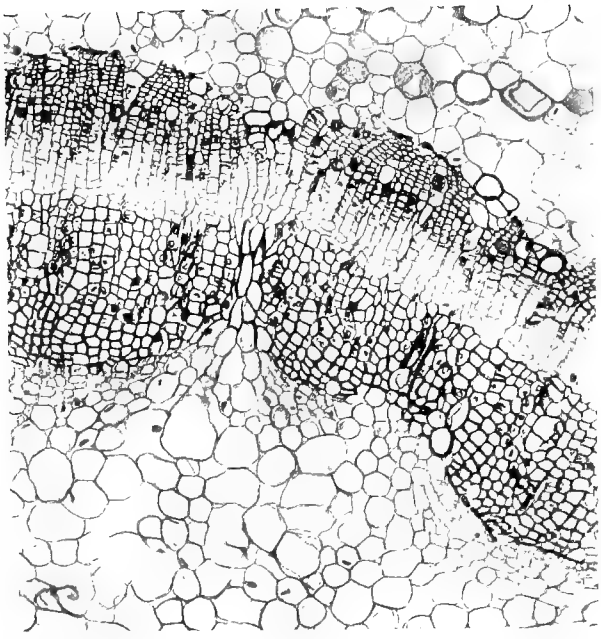
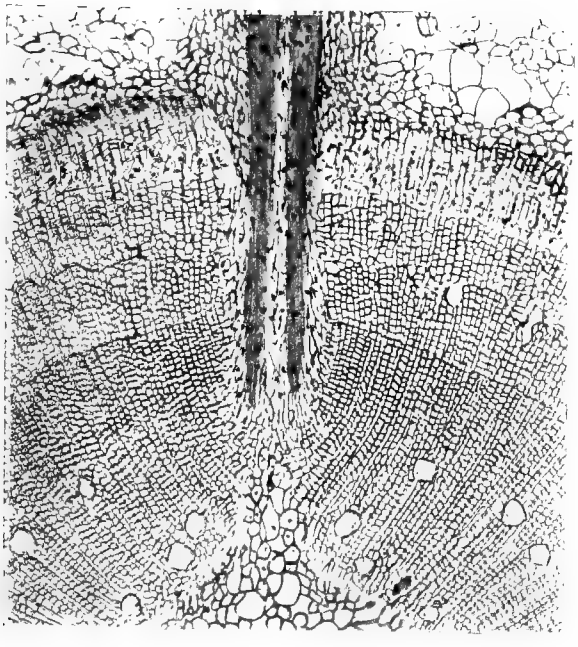








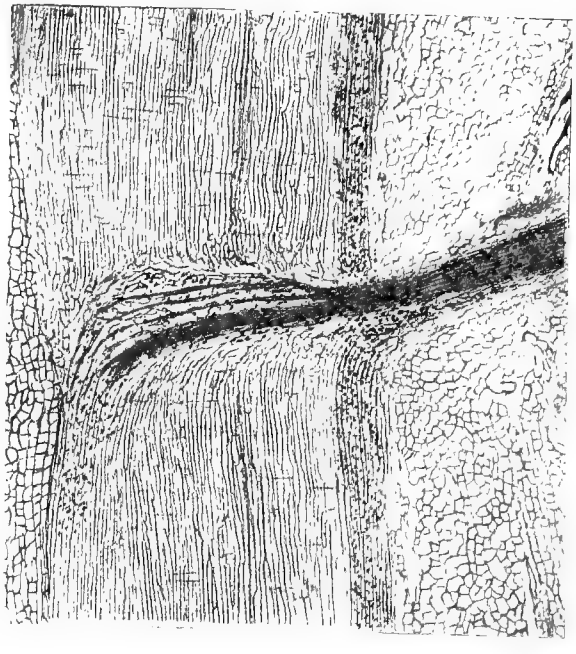


PLATE VII.

STEM STRUCTURE.

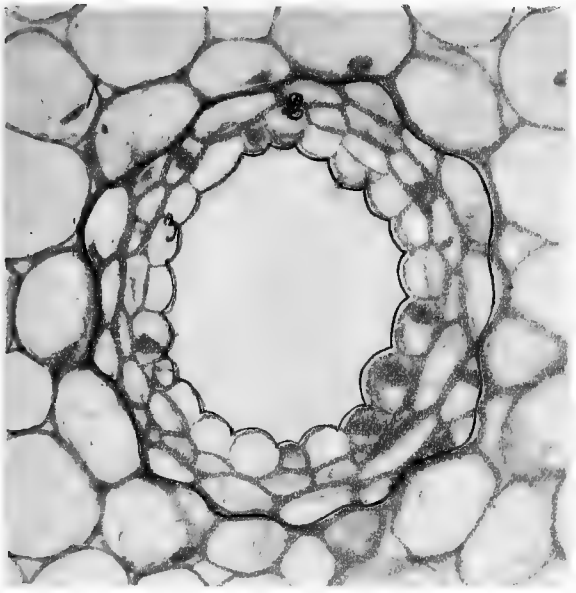
FIG. 23. Transverse section through resin passage. Stem of Pine × 75 dia. Tissue fixed as above. 1. Primary ground tissue. 2. Thickening band of prosenchyma. 3. Secreting cells of canal.

FIG. 24. Transverse section. Old Wood of Pine × 35 dia. Cut $^1/_{1000}$ in. Unstained. 1. Spring growth of the wood. 2. Autumn and winter growth. 3. Resin canal. 4. Medullary ray.

FIG. 25. Radial longitudinal section. Old Wood of Pine × 75 dia. Cut $^1/_{1000}$ in. Unstained. 1. Bordered pits in surface view. 2. Medullary ray in radial view. 3. Cell-wall in radial view.

FIG. 26. Tangential longitudinal section. Old Wood of Pine × 75 dia. Cut $^1/_{1500}$ in. 1. Bordered pits in transverse section. 2. Medullary ray in section. 3. Cell-wall in tangential view.

23



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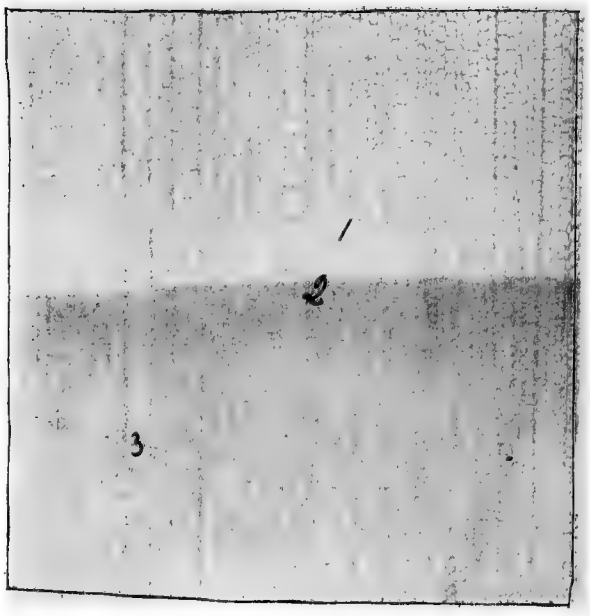

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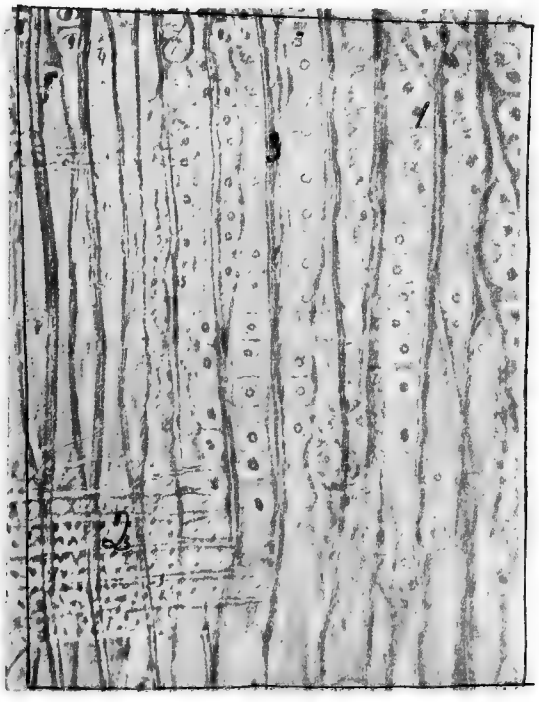

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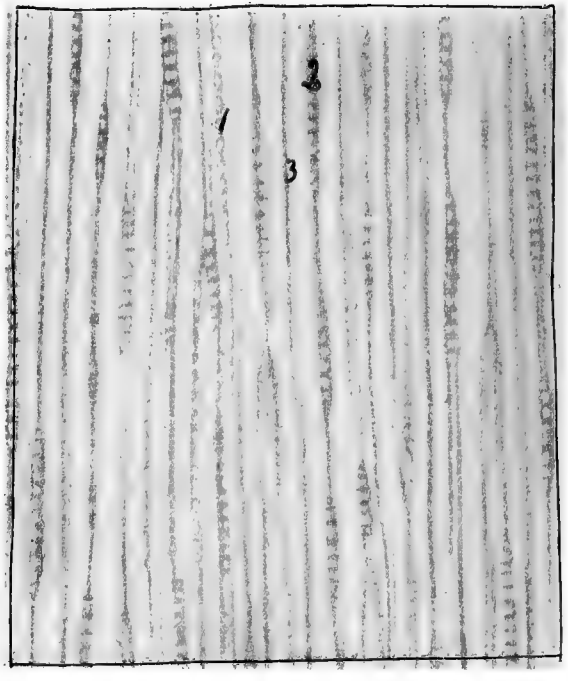
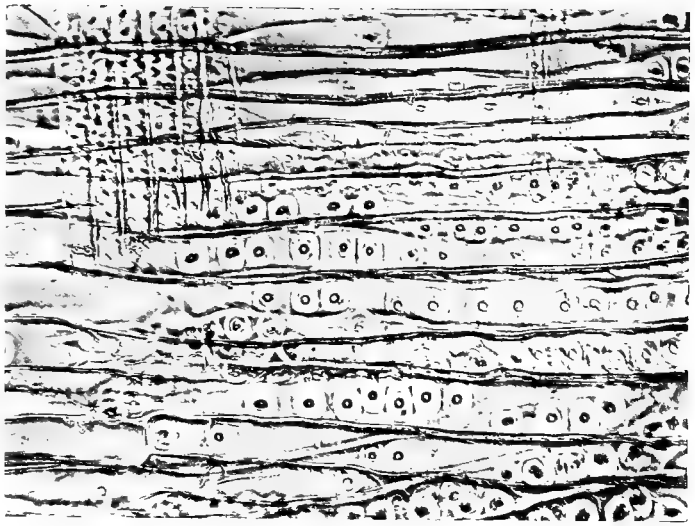
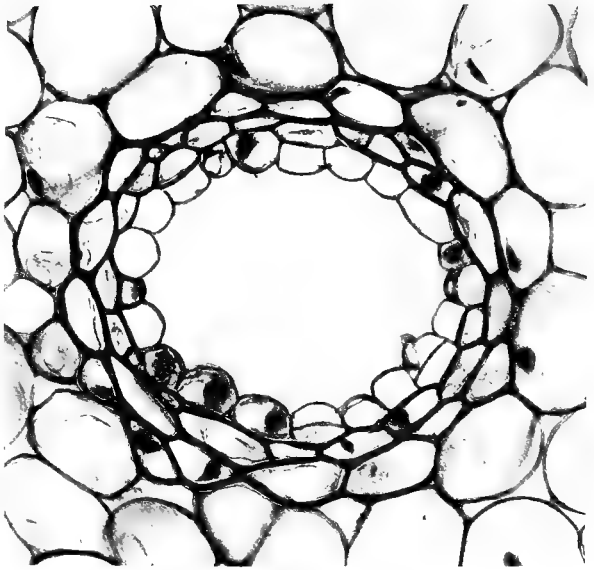
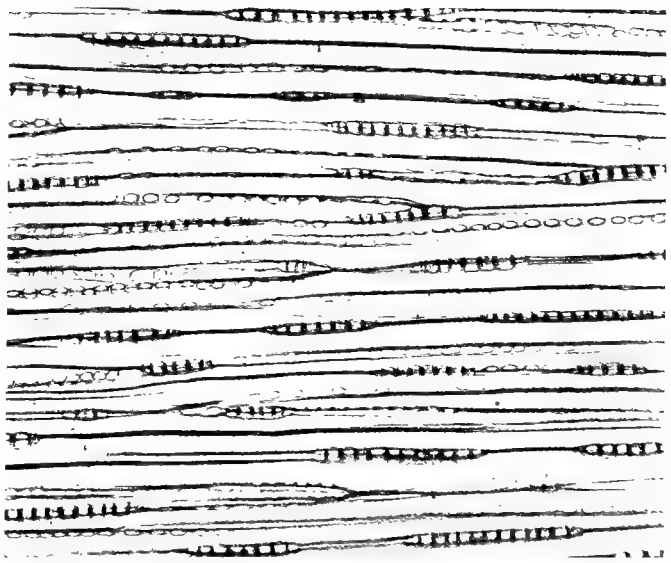








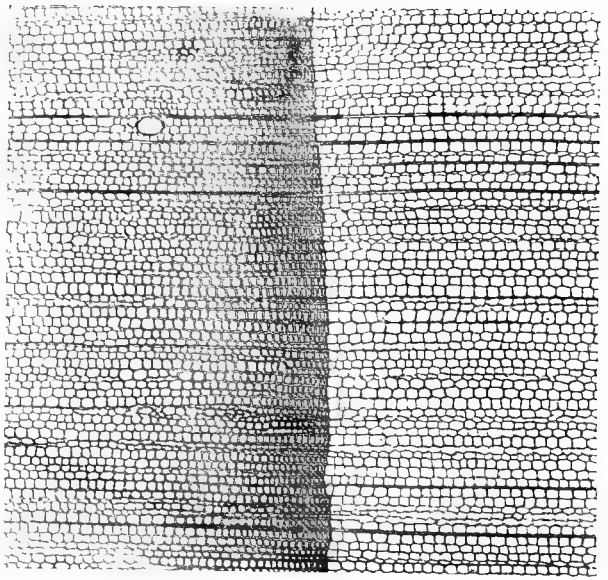


PLATE VIII.

STEM STRUCTURE.

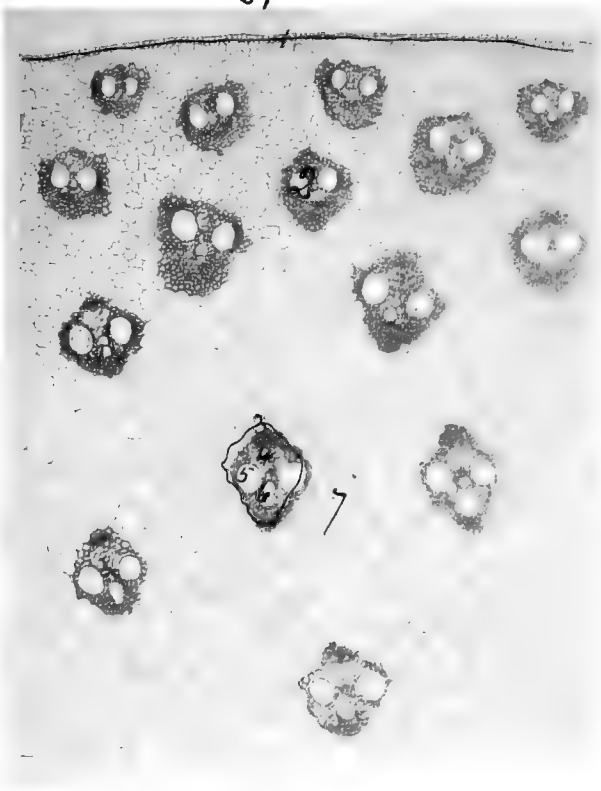
FIG. 27. Transverse section. Stem of Indian Corn. 'Zea mais' ×25 dia. Fixed in 92% Alcohol. Stained with Borax Carmine and Malachite Green. 1. Epidermal tissue. 2. Vascular bundle. 3. Bundle-sheath. 4. Phloem. 5. Large pitted vessel. 6. Annular vessel. 7. Primary ground tissue.

FIG. 28. Longitudinal section passing through a primary vascular bundle. Stem of Indian Corn. 'Zea mais' ×50 dia. Stained as in Fig. 27. 1. Primary ground tissue. 2. Large pitted vessels. 3. Annular vessel. 4. Spiral vessel.

FIG. 29. Transverse section through Rachis of Fern. 'Pteris aquilina' ×40 dia. Fixed in Chromo-Acetic solution. Staining as in above figures. 1. Primary ground tissue. 2. Endodermis. 3. Bast sheath. 4. Bast. 5. Scalariform vessels. 6. Spiral vessels.

FIG. 30. Longitudinal section of Rhizome of Fern. 'Pteris aquilina' ×60 dia. Fixed and stained as above. 1. Primary ground tissue. 2. Sclerenchyma sheath. 3. Bast. 4. Spiral vessels. 5. Scalariform vessels.

27



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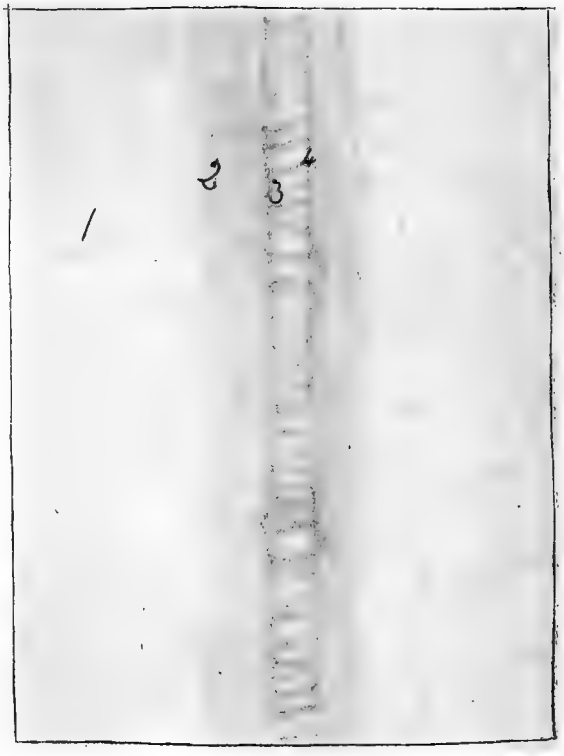

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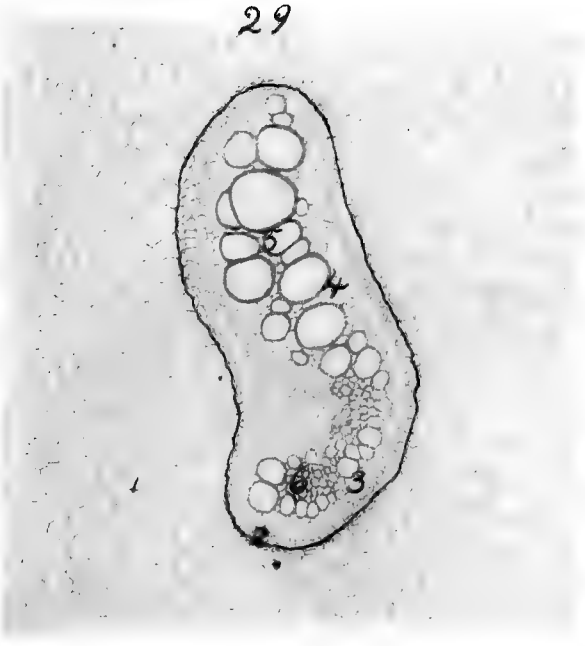

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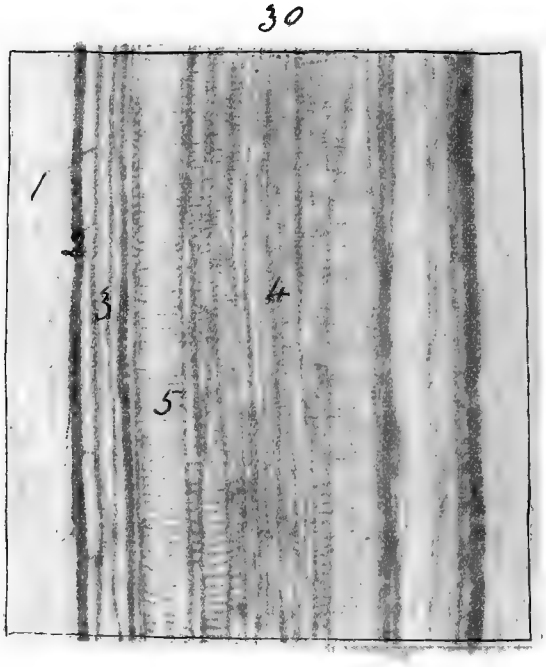

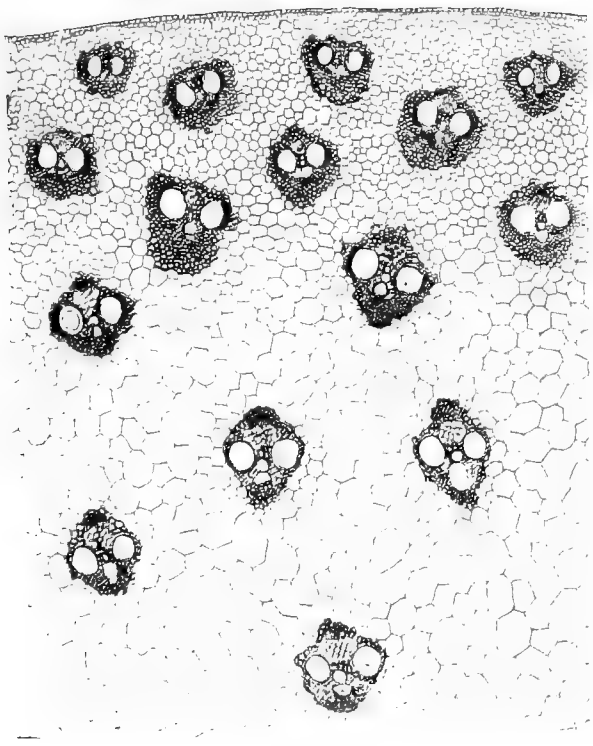

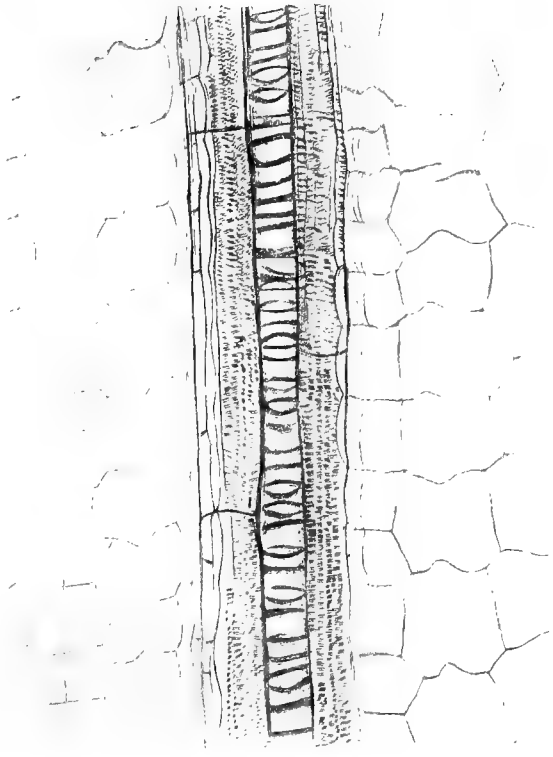

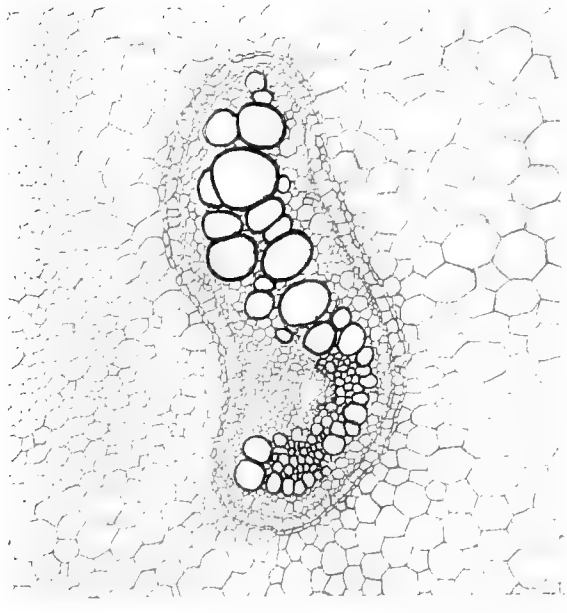

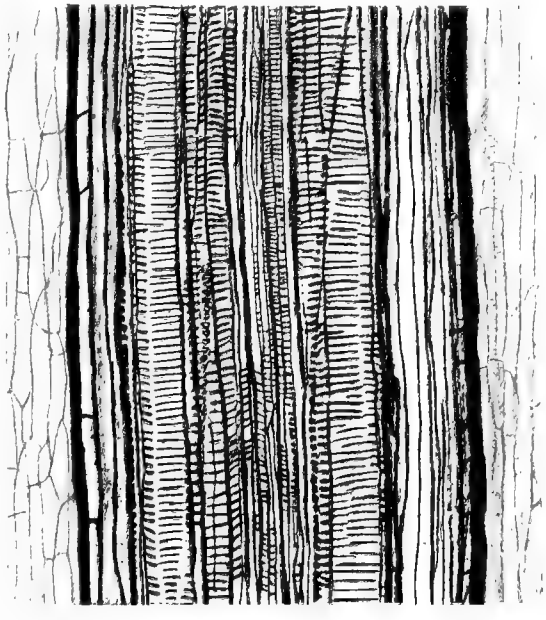

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PLATE IX.

STEM STRUCTURE.

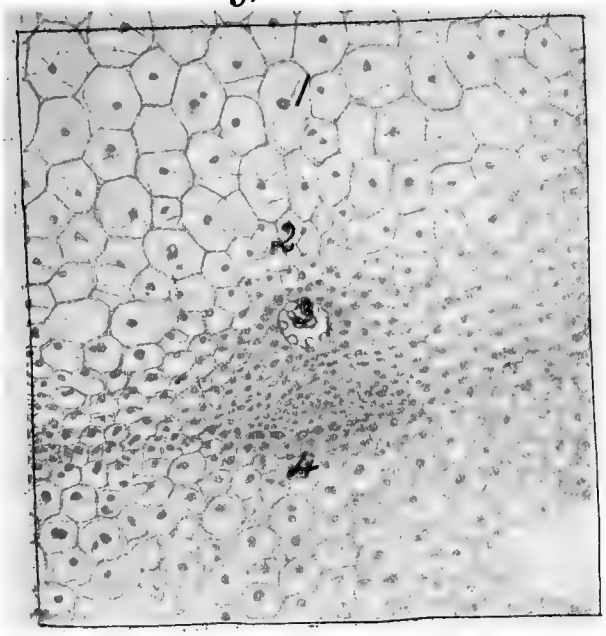
FIG. 31. Transverse section immediately below vegetative bud of 'Equisetum telmateia' × 75 dia. Tissue fixed in Chromo-Acetic solution. Stained with Borax Carmine and Malachite Green. 1. Nucleated cortical tissue. 2. Bast. 3. Isolated annular vessels in intercellular space. 4. Primary nucleated tissue.

FIG. 32. Transverse section through vegetative bud of 'Equisetum telmateia' × 10 dia. Fixed as above. Stained Carmine. 1. Central axis of stem. 2. Zone of vascular bundles. 3 and 4. Leaf-sheaths in various stages of development, the outer layer dividing into separate leaves.

FIG. 33. Longitudinal median section of an older vegetative bud than above × 30 dia. Fixed as above. Stained with Borax Carmine and Malachite Green. 1. Central ground tissue. 2. Nodal diaphragm. 3. Secretion cells. 4. Vascular system. 5. Lateral bud. 6. Cavity of vascular bundle. 7. Growing point of lateral bud. 8. Caniculi in formation.

FIG. 34. Longitudinal median section through growing point of lateral bud in Fig. 33 (7). 1. Meristem tissue. 2. Bud sheath. 3. Apical cell. 4. Zone of dividing nucleated tissue. Cut $^1/_{1000}$ in. Stained Brazilin × 120 dia.

31



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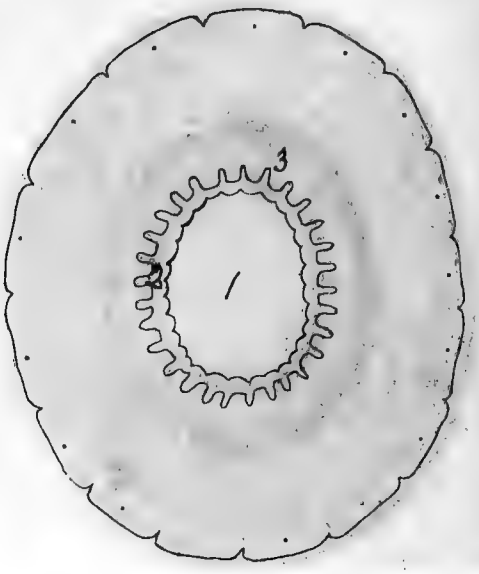

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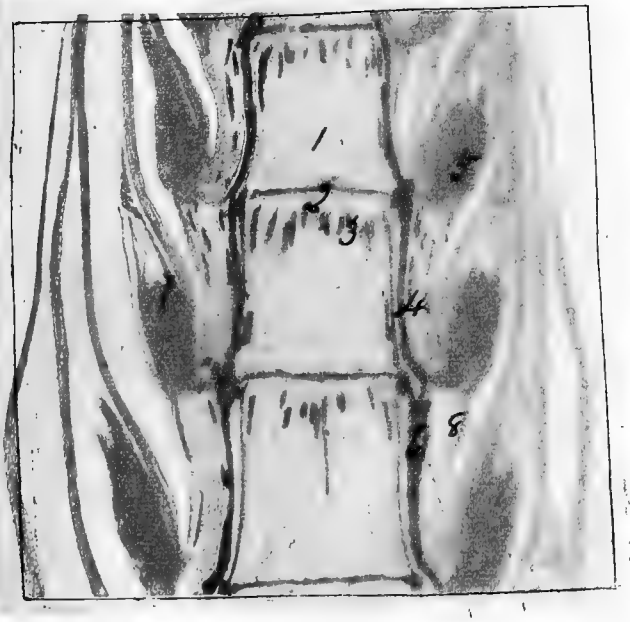

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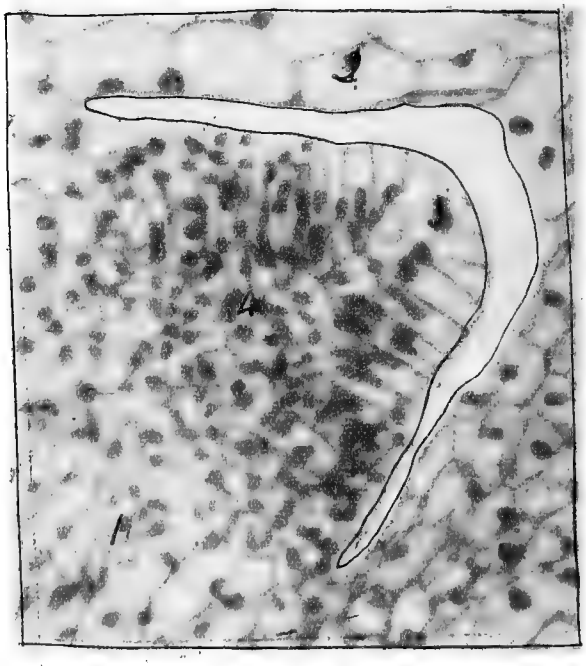
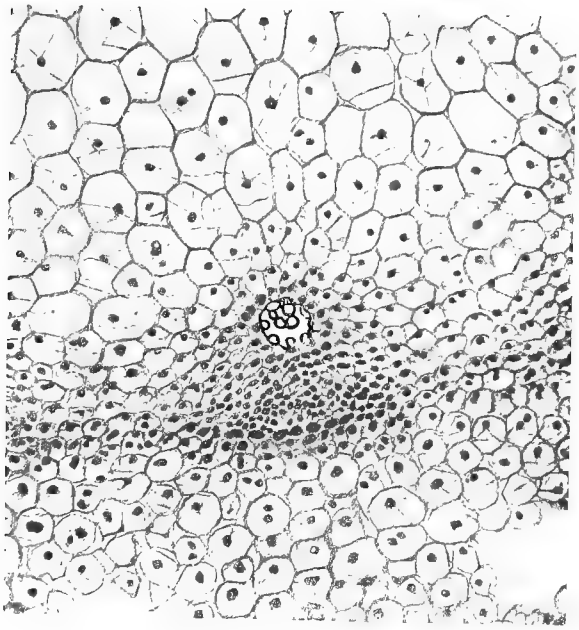
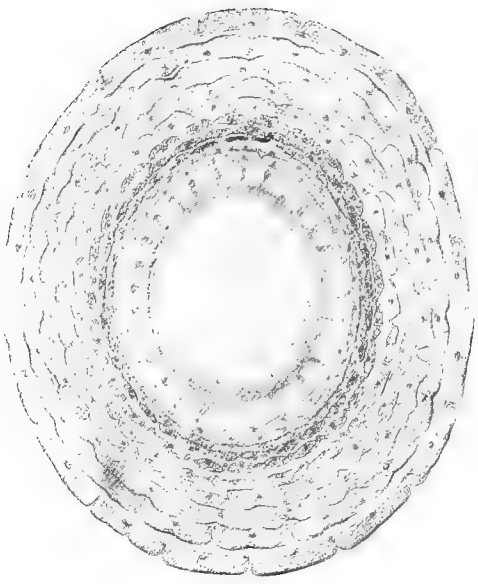
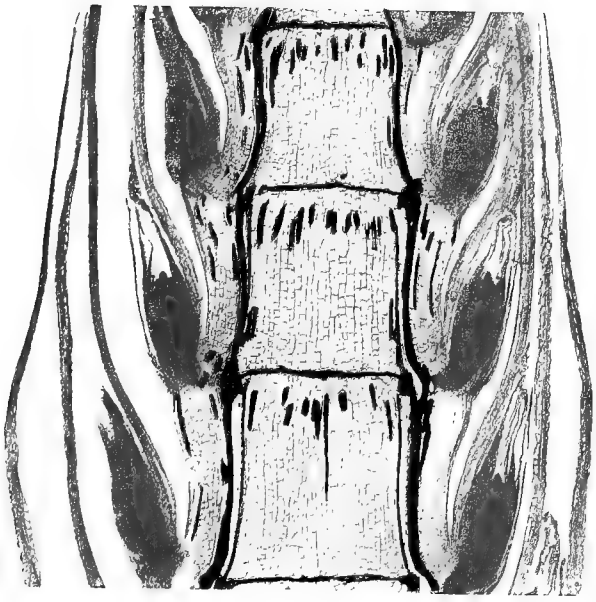








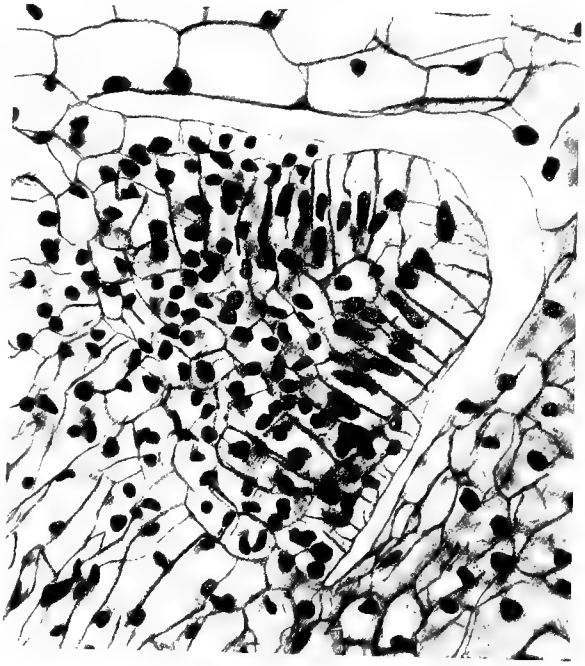


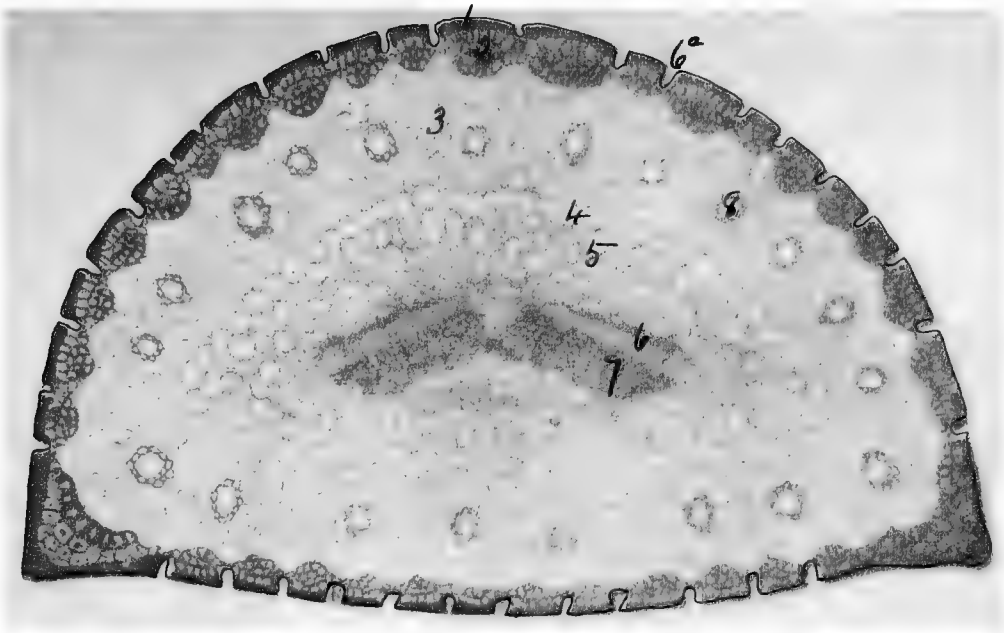
PLATE X.

LEAF STRUCTURE.

FIG. 35. Transverse section through Leaf of Pine. 'Pinus maritimus' ×45 dia. Fixed in 92% Alcohol. Stained with Borax Carmine and Malachite Green. 1. Epidermis. 2. Hypoderm. 3. Assimilating tissue. 4. Endodermis. 5. Transfusion tissue. 6. Phloem. 6a. Stomata. 7. Xylom. 8. Resin duct.

FIG. 36. Transverse section through Leaf of Marram Grass. 'Ammophila arundiacea' ×35 dia. Fixed and stained as above. 1. Epidermis. 2. Assimilating tissue. 3. Wood vessels. 4. Stiff leaf hairs. 5. Phloem of bundle. 6. margin of leaf.

35



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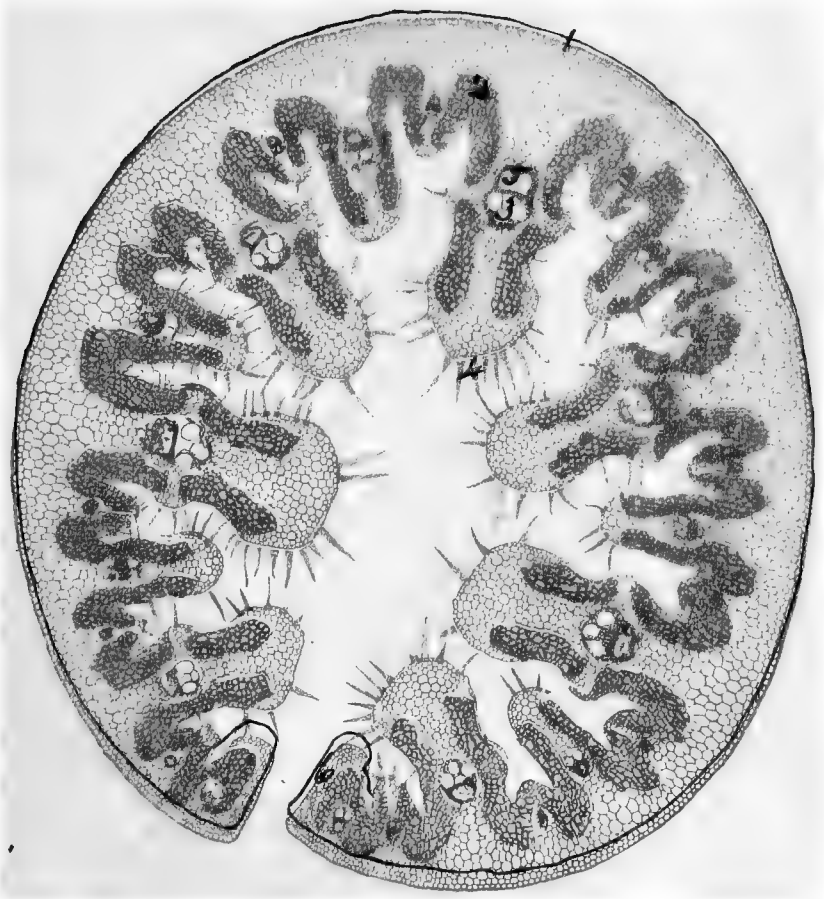
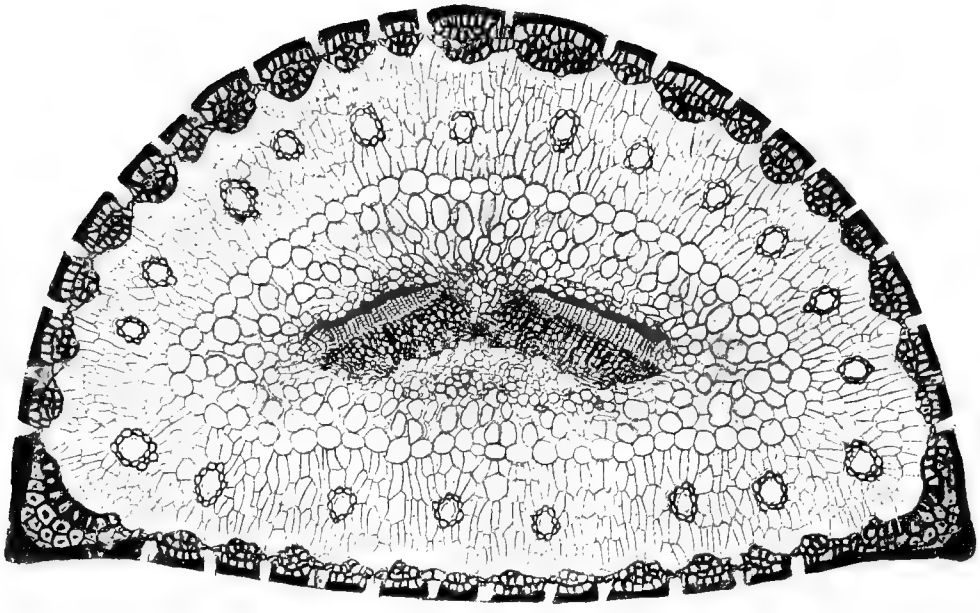




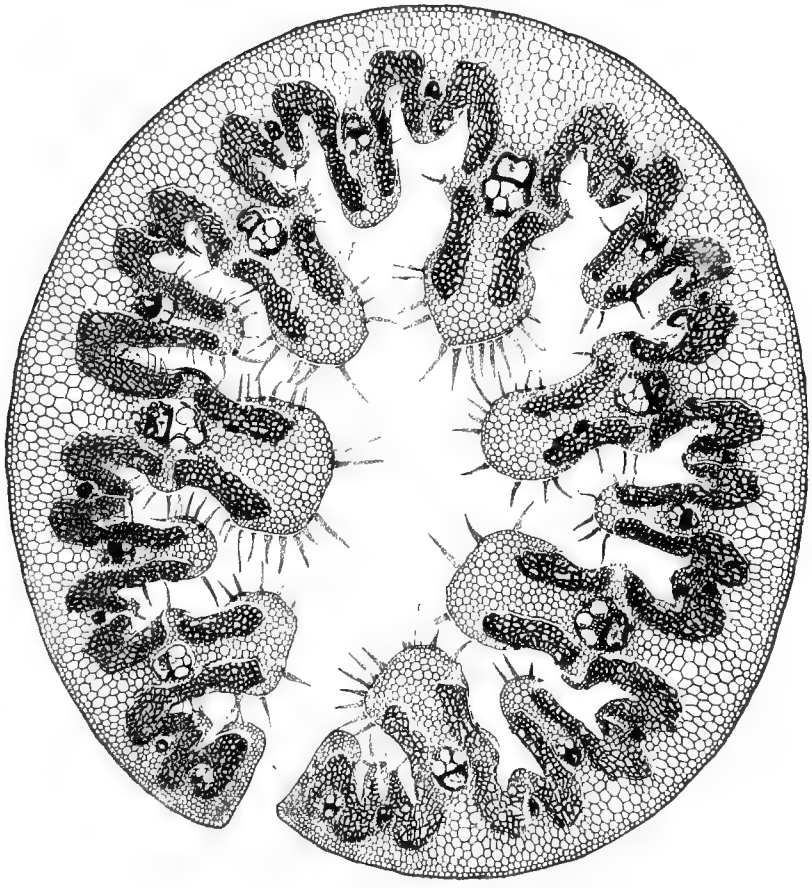


PLATE XI.

LEAF STRUCTURE.

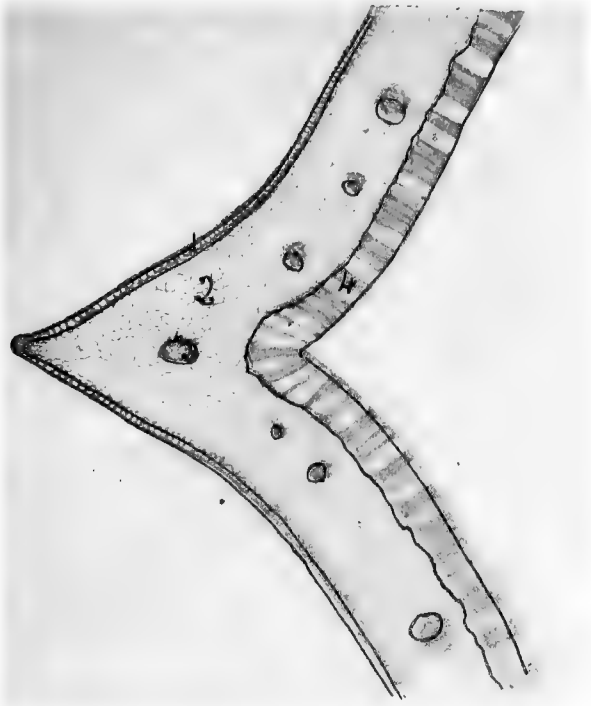
FIG. 37. Transverse section. Leaf of 'Cypripedium sp' × 20 dia. Fixed in 92% Alcohol. Stained with Borax Carmine and Malachite Green. 1. Lower epidermis of leaf. 2. Primary ground tissue. 3. Vascular bundle. 4. Water storage cells of upper epidermis, having their upper and under walls cut away.

FIG. 38. Transverse section. Leaf of Cycas. 'Cycas revoluta × 35 dia. Fixed with the Chromo-Acetic solution. Stained with Aniline-picrate and Gossypimine. 1. Lower epidermis of leaf. 2. Ground tissue. 3. Sclerenchymatous fibres. 4. Bast elements of bundle. 5. Phloem elements. 6. Stomata. 7. Palisade layer containing chloroplasts, stained blue.

FIG. 39. Transverse section. Leaf of Bottle-brush. 'Metrosideros verni' × 60 dia. Fixed with Chromo-Acetic solution. Cut $^1/_{2688}$ in. Stained with Aniline-picrate and Gossypimine. 1. Palisade layer, continuous on upper and under side. 2. Stomata. 3. Guard cells. 4. Primary ground tissue. 5. Xylem elements of bundle. 6. Phloem elements. 7. Small vascular bundles. 8. Oil gland.

FIG. 40. Part of Fig. 39 × 160 dia. 1. Cuticle. 2 and 3. Stoma and guard-cell. 4. Primary vascular bundle. 5. Vascular bundle in longitudinal section. 6. Palisade cells containing chloroplasts, stained blue.

37



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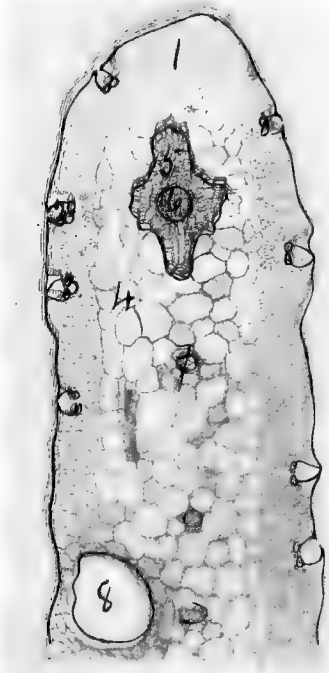

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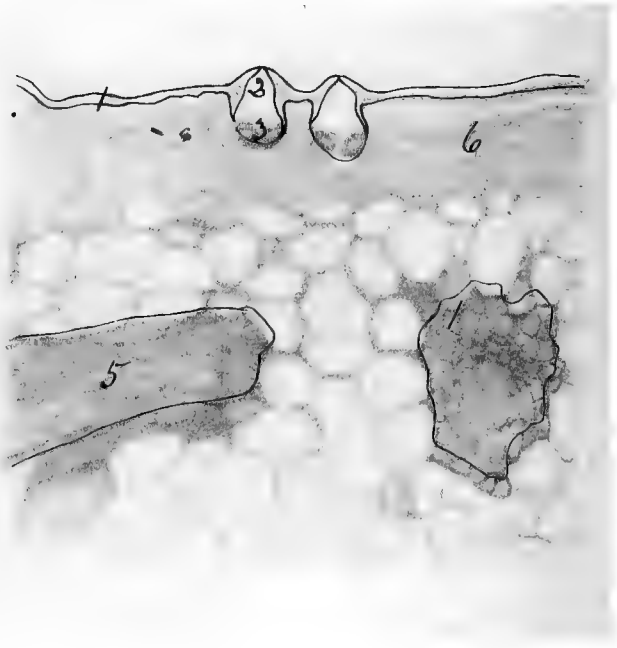

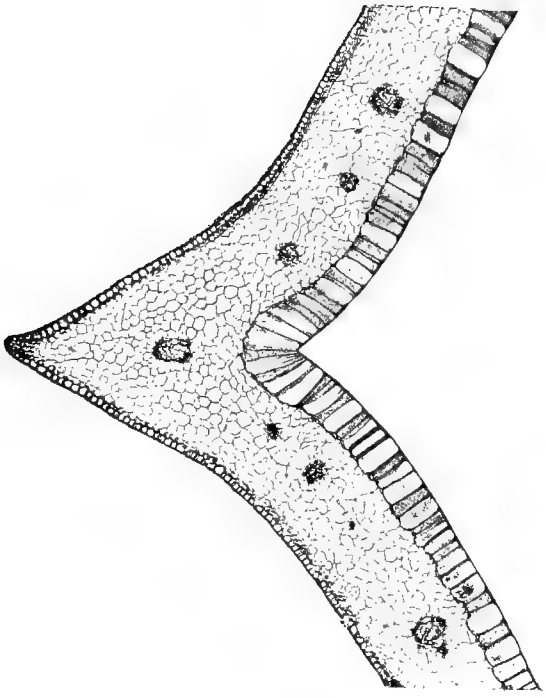

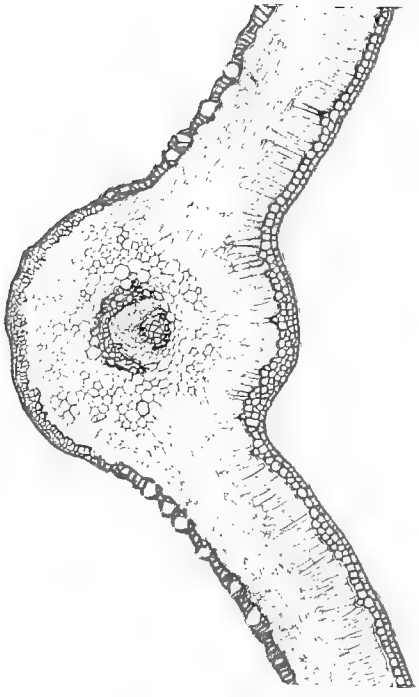

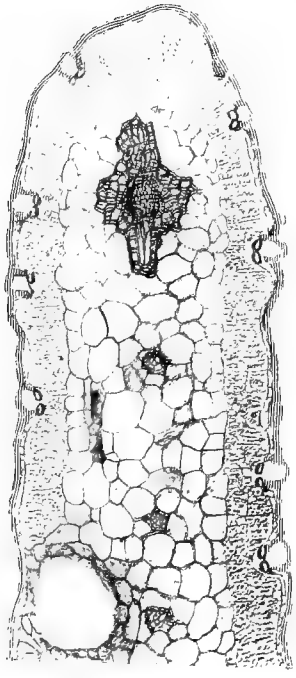

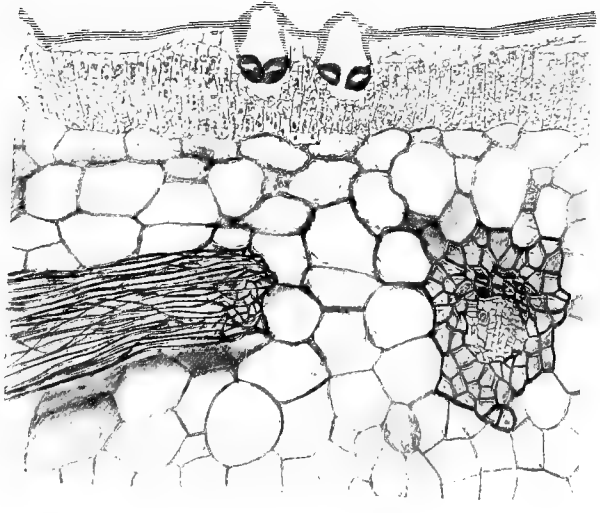

PLATE XII.

LEAF STRUCTURE.

FIG. 41. Transverse section. Leaf of Tradescantia. 'Tradescantia discolor' × 35 dia. Fixed with Chromo-Acetic solution. Stained with Aniline-picrate and Gossypimine. 1. Lower epidermis. 2. Primary ground tissue. 3. Dense layer of assimilating tissue. 4. Vascular bundle. 5. Air cavity. 6. Stoma. 7. Upper epidermis, showing vertical walls of the cells only. 8. Mucilaginous lining of the cell. 9. Nucleus of cell. 10. Upper cuticle of leaf.

FIG. 42. Cuticle. Leaf of Monkey-puzzle. 'Araucaria imbricata' × 50 dia. Stained with Brazilin. 1. Cuticularised tissue. 2. Stomata.

FIG. 43. Transverse section. Green Stem of 'Casuarina equisetifolia' × 50 dia. Fixed in Chromo-Acetic solution. Stained with Borax Carmine and Malachite Green. 1. Cortical tissue. 2. Leaf-trace bundles. 3. Cambium layer. 4. Xylem elements. 5. Primary medullary ray. 6. Central axis of stem. 7. Stomata bordering the stem cavities.

FIG. 44. Epidermis and stinging-hairs from midrib of young Leaf of Nettle. 'Urtica dioica' × 35 dia. Fixed with Chromo-Acetic solution. Stained with Borax Carmine and Malachite Green. 1. Cuticularised epidermis of midrib. 2. Enlarged base of sting. 3. Cavity of sting, filled with irritant fluid. 4. Capitate apex of sting. 5. Stiff hairs of midrib.

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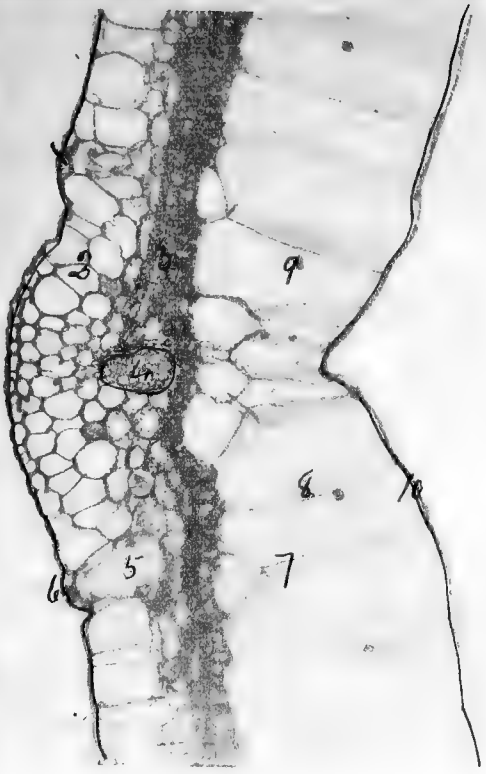

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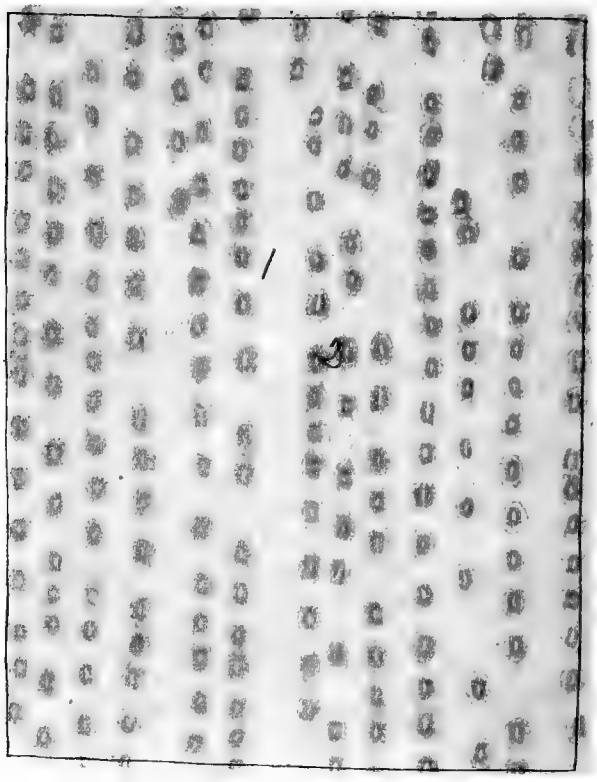

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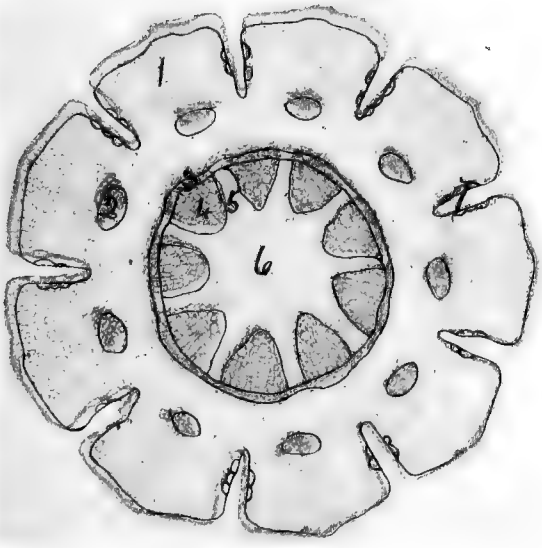

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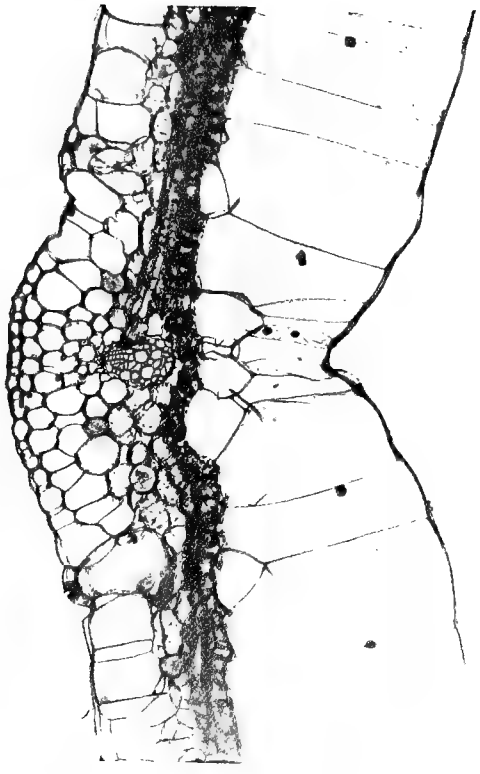

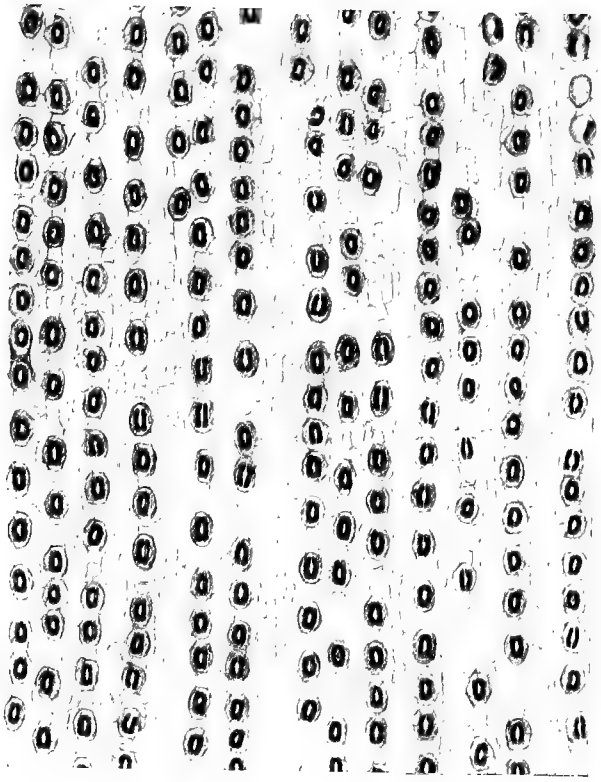

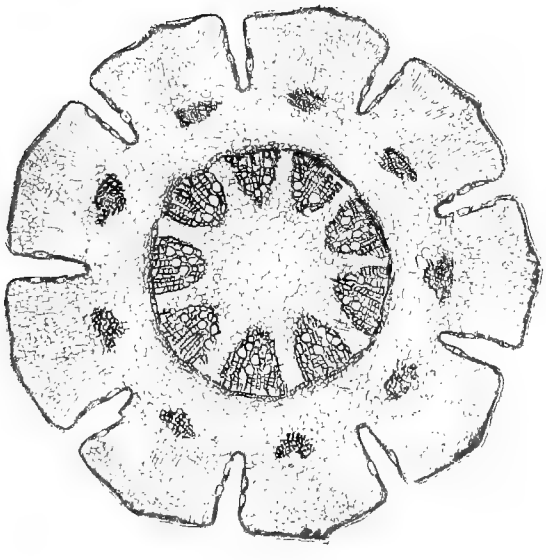

PLATE XIII.

LEAF STRUCTURE.

FIG. 45. Transverse section. 'Phyllode.' 'Acacia decurrens' × 60 dia. Fixed with Chromo-Acetic solution. Stained with Aniline-picrate and Gossypimine. 1. Epidermis. 2. Stoma. 3. Palisade layer, containing chloroplasts, stained blue. 4. Vascular bundle. 5. Smaller vascular bundles—*a*, xylem elements of bundle; *b*, cambium; *c*, phloem, all having become lignified.

FIG. 46. Transverse section. 'Cladode' of 'Ruscus aculeatus' × 35 dia. Fixed with Chromo-Acetic solution. Stained with Aniline-picrate and Gossypimine. 1. Epidermis. 2. Ground tissue (spongy parenchyma). 3. Primary vascular bundle. 4. Stoma. 5. Smaller vascular bundle.

FIG. 47. Transverse section. Leaf Bud of Beech Tree. 'Fagus sylvatica' × 25 dia. Fixed with Chromo-Acetic solution (Celloidinised preparation). Stained with Borax Carmine. 1. Protective bracts or scales. 2. Internal bud scales. 3—7. Complete young leaf in section—3, leaf-blades; 4, midrib; 5, 6, 7, lateral veins.

FIG. 48. Transverse section. Leaf Bud of Ash Tree. 'Fraxinus excelsior' × 25 dia. Fixed with Chromo-Acetic solution. Stained with Borax Carmine. 1.—2. Imbricate bud scales. 3. Petiole of oldest leaf, leaf-blade being cut away in previous sections. 4. Vascular system of same. 4a. Petiole of secondary leaf, the complete leaf inclosed in dotted outline. 6—7. Developing leaves.

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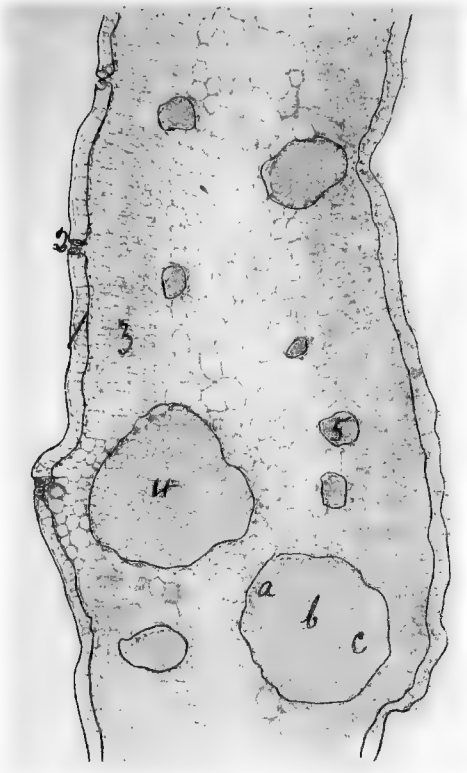

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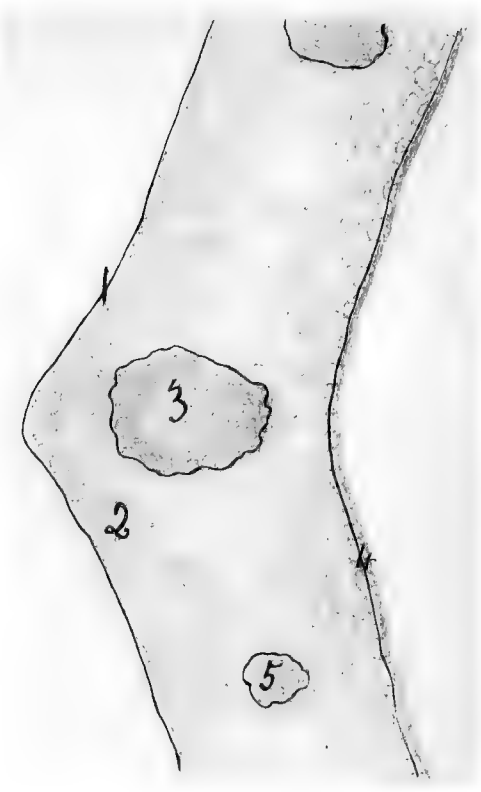

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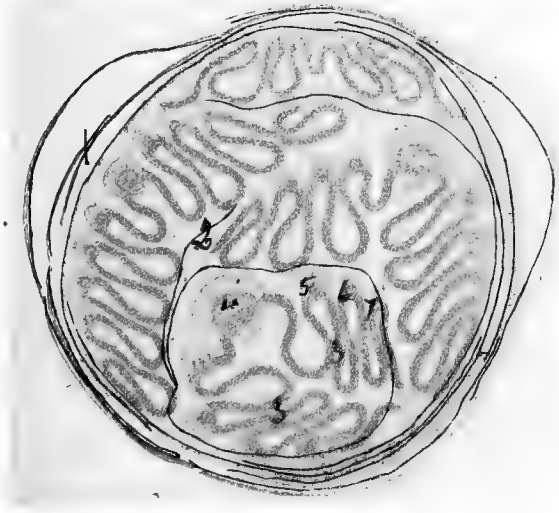

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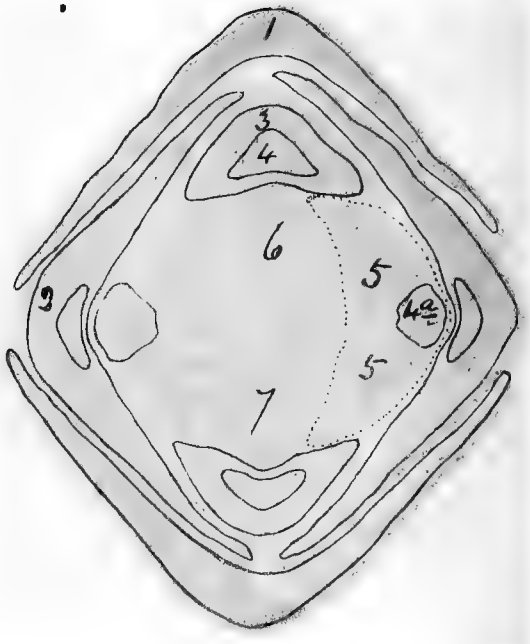
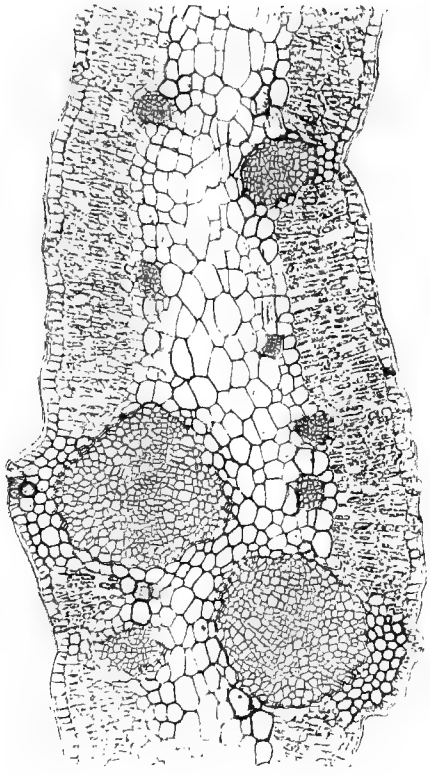
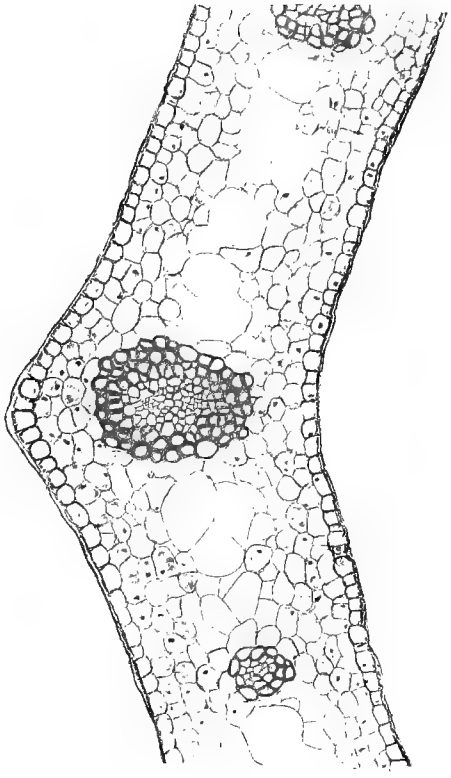






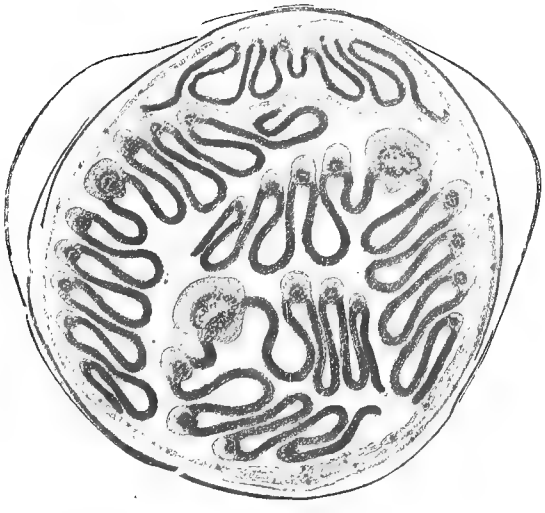


PLATE XIV.

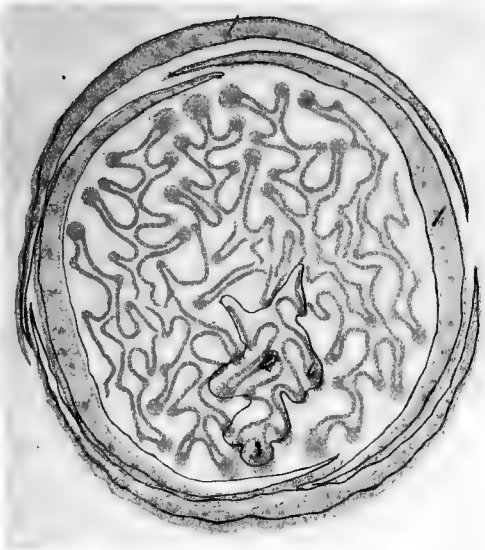
LEAF STRUCTURE.

FIG. 49. Transverse section. Leaf Bud of Sycamore. 'Acer pseudo-platanus' × 25 dia. (Celloidinised preparation). Stained Borax Carmine. 1. Imbricate bud scales. 2. Developing leaf 'outlined.' 3. The midrib. 4. Lateral veins of leaf.

FIG. 50. Longitudinal median section of lateral branch of Sycamore, passing through Leaf Stalk of current year and Developing Bud of next year × 16 dia. Fixed in 92% Alcohol. Left half only of the section is shown. 1. Pith. 2. Xylem elements of branch. 3. Cambium. 4. Phloem. 5. Cortical tissue. 6. Central axis of developing branch. 7. Zone of meristem tissue. 8. Bud scales. 9. Leaves in development. 10. Xylem of developing branch. 11. Petiole of leaf. 12. Ground tissue of same. 13. The vascular system. 14. The separating or absciss layer,' which forms during the period of vegetation, and through which 'defoliation' takes place in Autumn (leaf-fall).

FIG. 51. Longitudinal median section of a complete Branch of Sycamore, showing the position of the opposite petioles of the current year and the axillary buds for next year's vegetative period. 1. Central axis (pith). 2. Petiole of current year. 3. Central axis of next year's branch. 4. Separating layer. 5. Vascular bundle leading to leaf. 6. Xylem elements of branch. 7. Cambium. 8. Phloem. 8a. Cortical tissue. 9. Bud Scales. 10. Developing leaves. 11. Growing point of branch. The action of separation has taken place from the point marked X.

49



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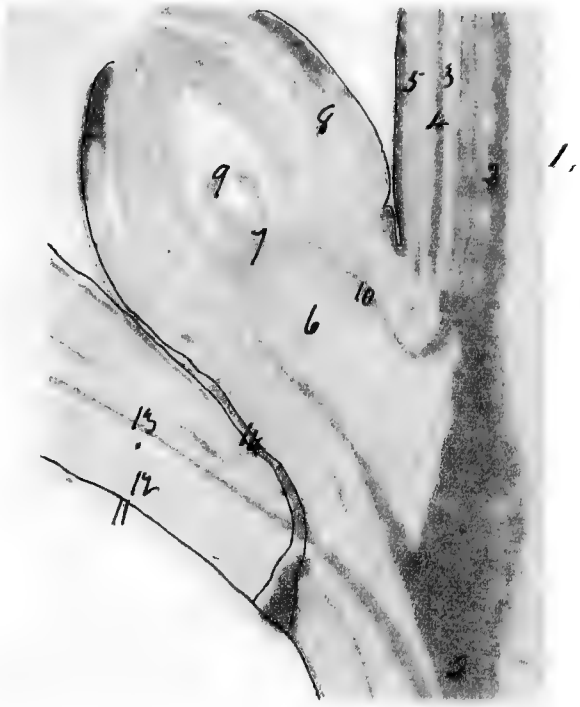

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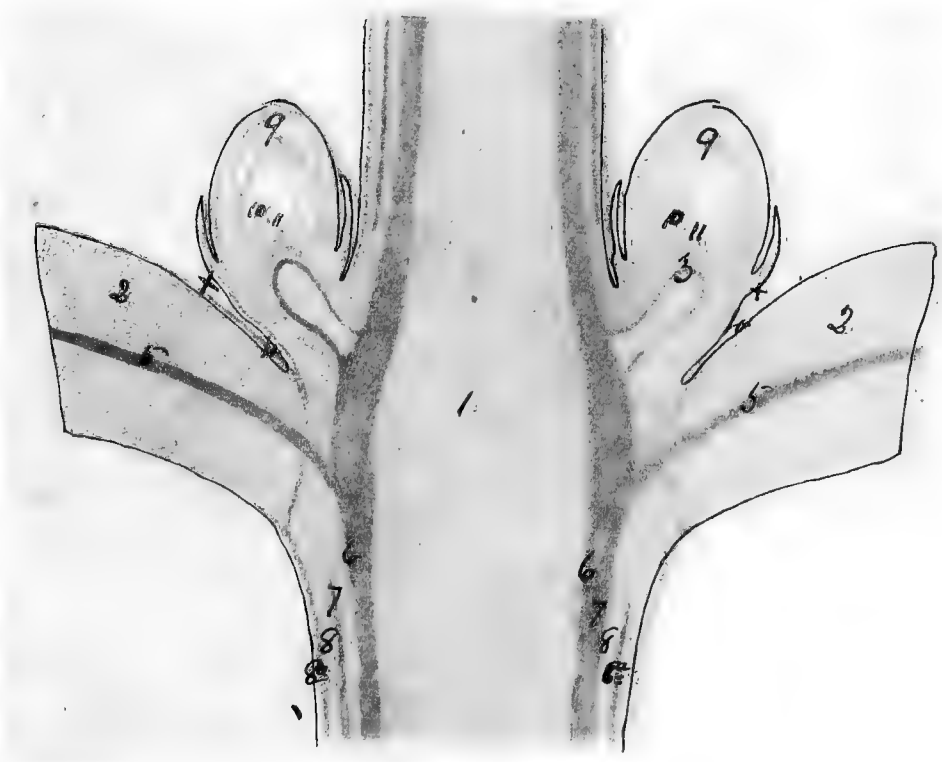
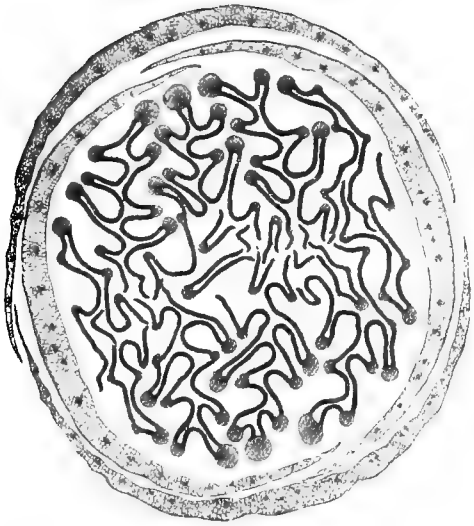




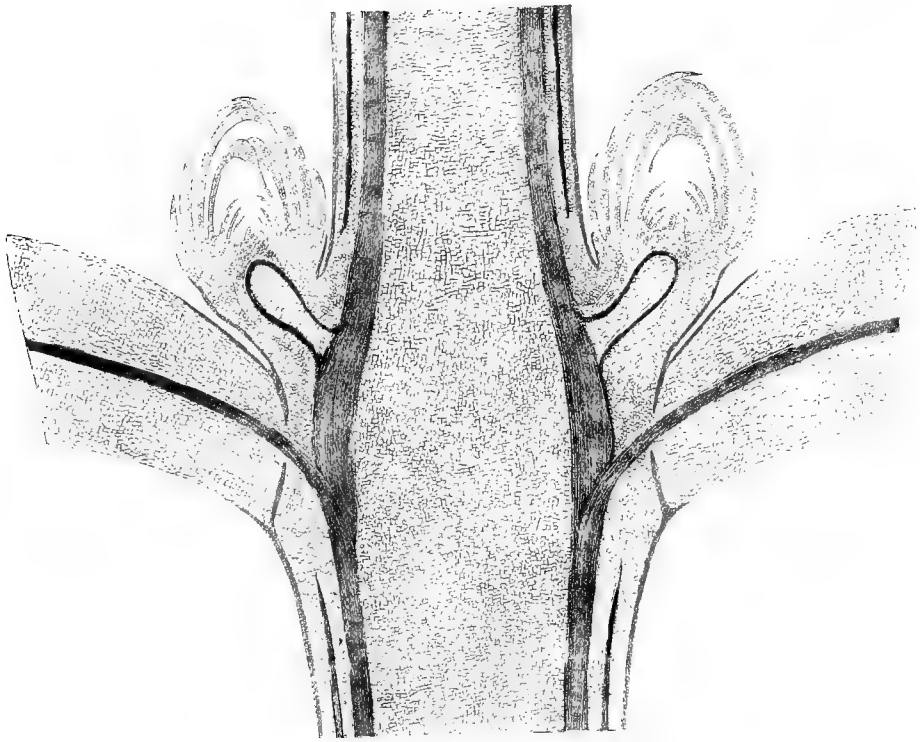


PLATE XV.

FLORAL STRUCTURE.

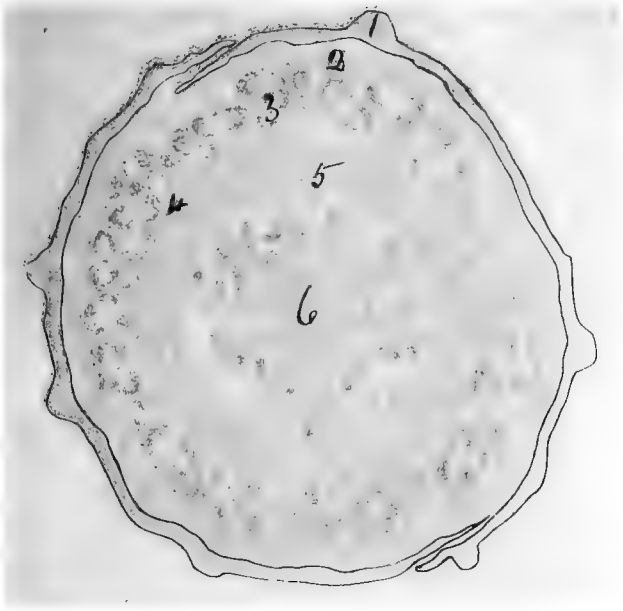
FIG. 52. Transverse section. Flower Bud of Poppy. 'Papaver rhœas' × 15 dia. Fixed in 92% Alcohol (Celloidinized preparation). Stained with Borax Carmine. 1. Calyx. 2. Corolla. 3. Anthers containing pollen. 4. Base of Stigmatic Cap. 5. Wall of Ovary. 6. Placenta.

FIG. 53. Transverse section. Flower Bud of Wallflower. 'Cheiranthus Cheiri' × 25 dia. Fixed in 92% Alcohol. Stained with Borax Carmine (Celloidinized preparation). 1. Calyx. 2. Corolla. 3. Anthers. 4. Capsule (Silique).

FIG. 54. Transverse section. Flower Bud of Dandelion. 'Taraxacum officianalis' × 35 dia. Fixed with Chromo-Acetic solution (Celloidinized preparation). Stained with Borax Carmine. 1. Bracts of the involucrum. 2. Pappus (modified calyx). 3 Tubular corolla. 4. Five 'syngenesious' anthers. 5. Style with three vascular bundles.

FIG. 55. Transverse section. Flower Bud of Iris. 'Iris Germanica' × 25 dia. Fixed with 92% Alcohol (Celloidinized preparation). Stained with Borax Carmine. 1. Calyx. 2. Corolla. 3. Petaloid stigmas. 4. Anthers. 5. Fringe of hairs running longitudinally along the median plane of each sepal.

52



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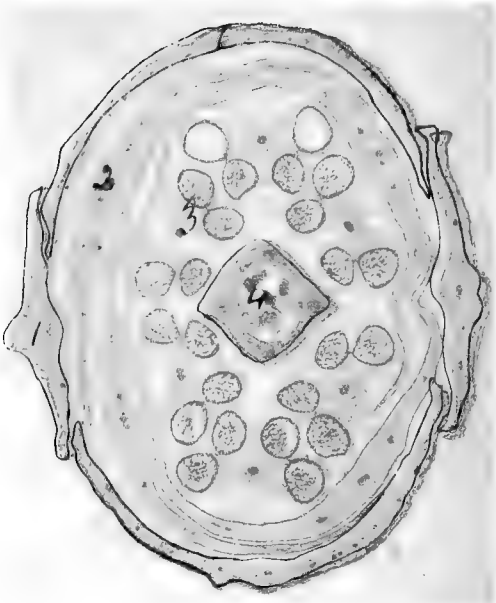

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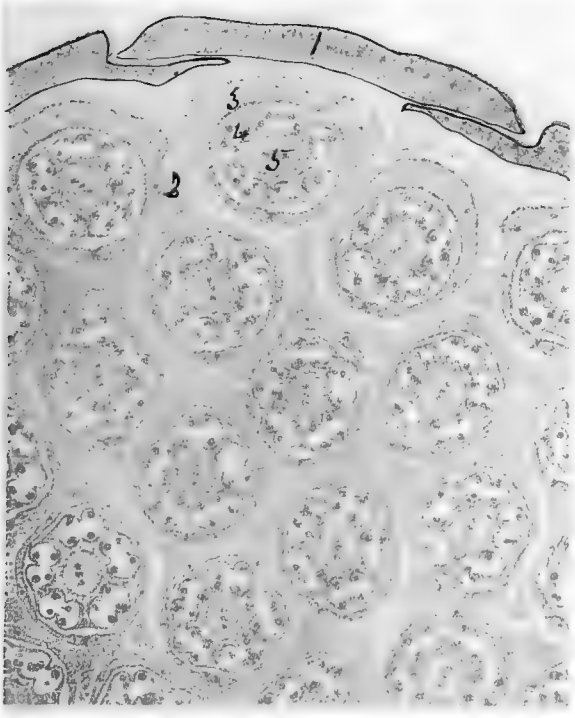

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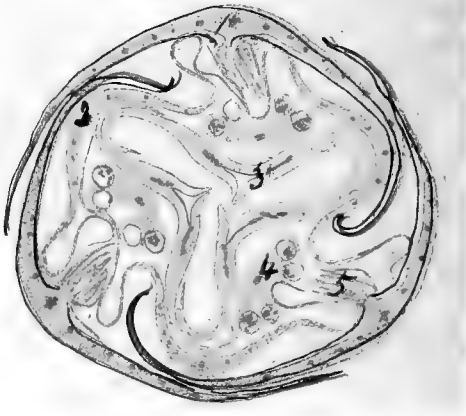

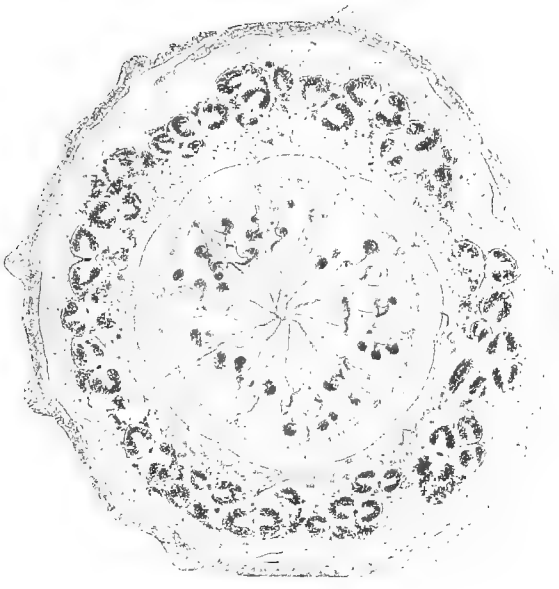

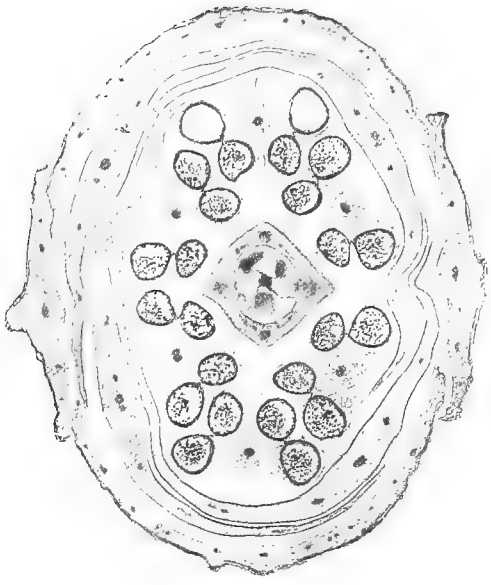

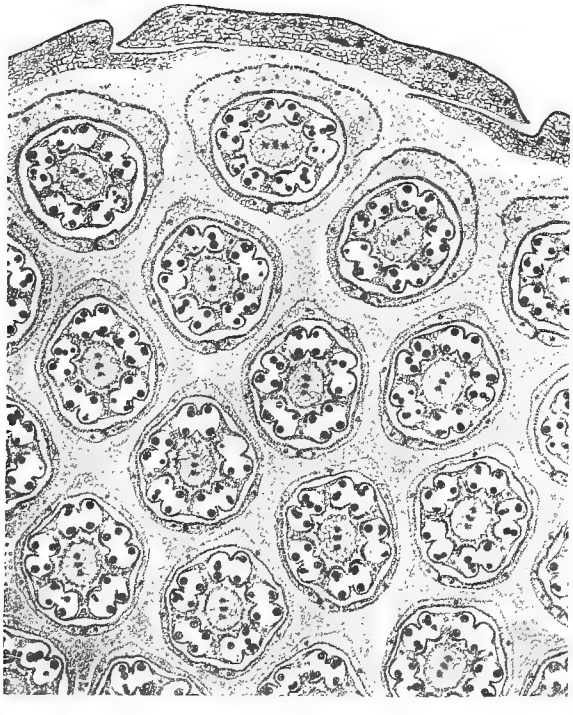

PLATE XVI.

FLORAL STRUCTURE.

FIG. 56. Transverse section. Flower Bud of Lily. 'Lilium croceum' ×10 dia. Fixed with Chromo-Acetic solution. Stained with Borax Carmine. (Celloidinised preparation.) 1. Calyx. 2. Corolla. 3. Anthers. 4. Ovary.

FIG. 57. Transverse section of an Anther Lobe of Lily. 'Lilium croceum' ×60 dia. Fixed with Chromo-Acetic solution. Stained with Brazilin. 1. Fibrous layer. 2. Zone of smaller nucleated cells. 3. Tapetal layer. 4. Pollen grains.

FIG. 58. Transverse section. Ovary of Lily. 'Lilium croceum' ×75 dia. Fixed with Chromo-Acetic solution. Stained with Brazilin. 1. Outer wall of ovary. 2. Inner wall. 3. Cavity of ovary. 4. Embryo-sac. 5. Central axis.

FIG. 59. Longitudinal median section. Catkin of Sallow. 'Salix caprœa' ×15 dia. Fixed with Chromo-Acetic solution. Stained with Aniline-picrate and Gossypimine. 1. Central axis (pith). 2. Vascular system. 3. Anthers. 4. Floral Scales (bracts). 5. Nectary.

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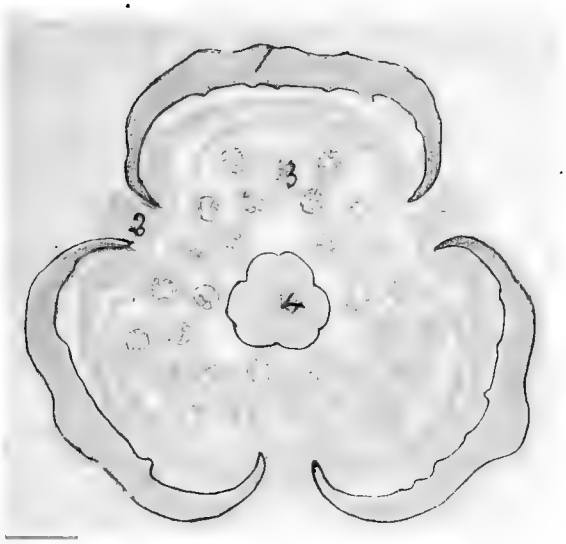

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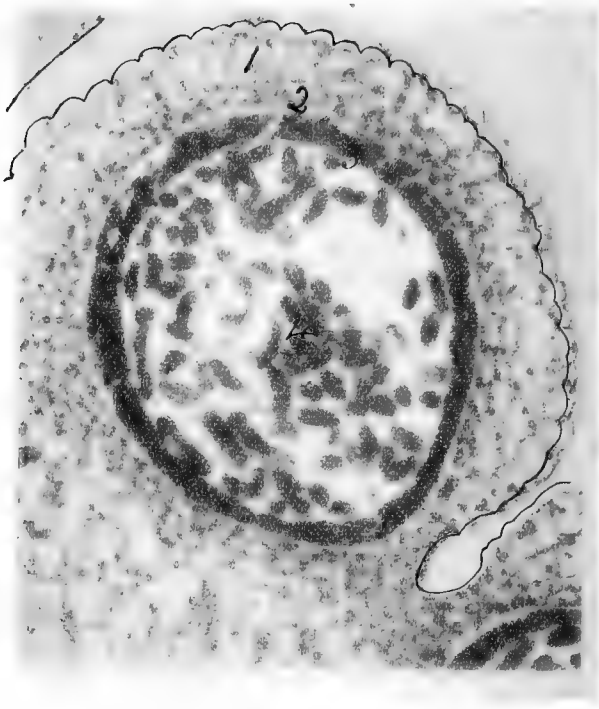

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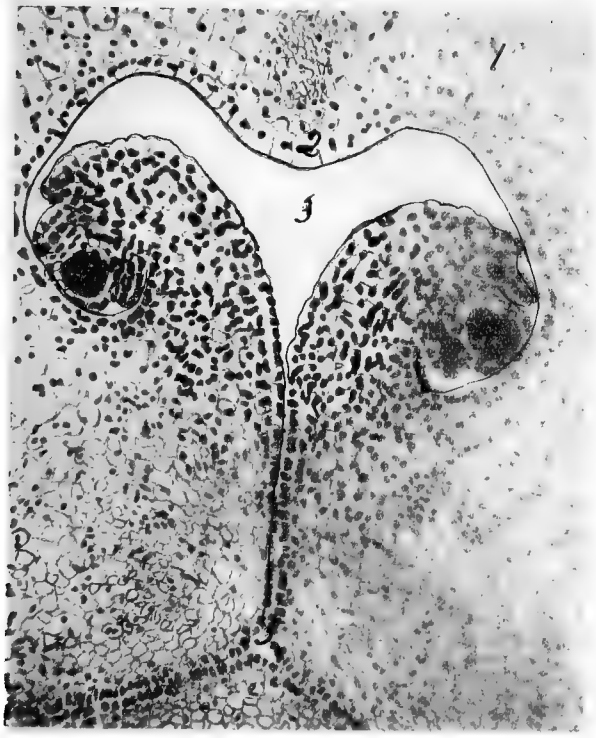

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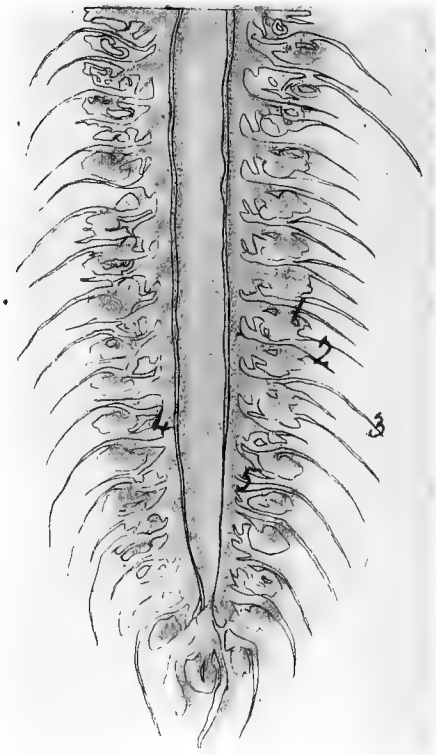

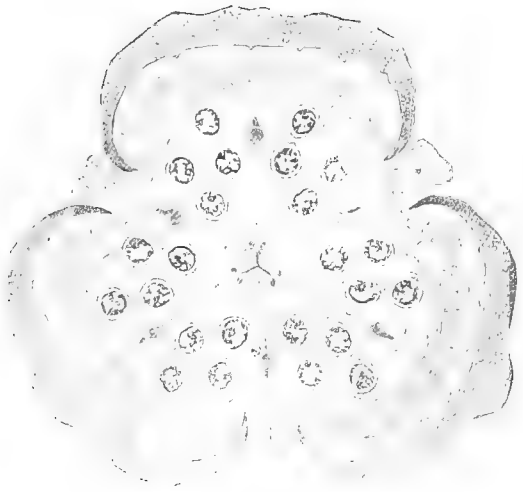

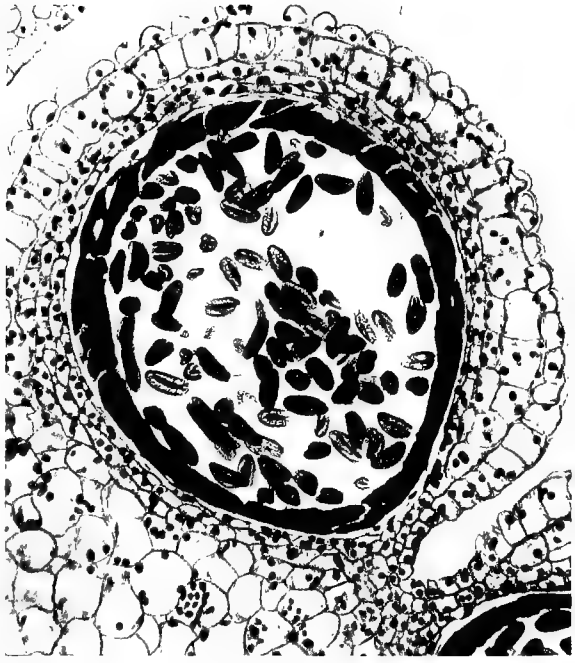

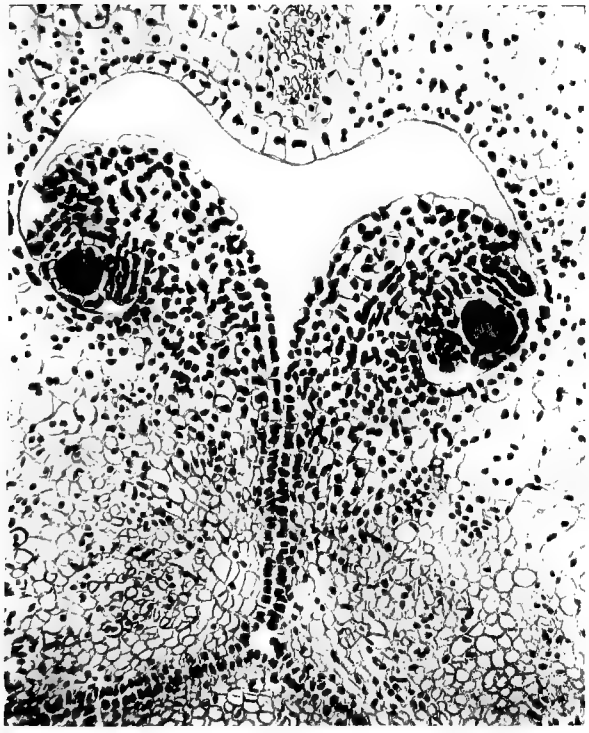

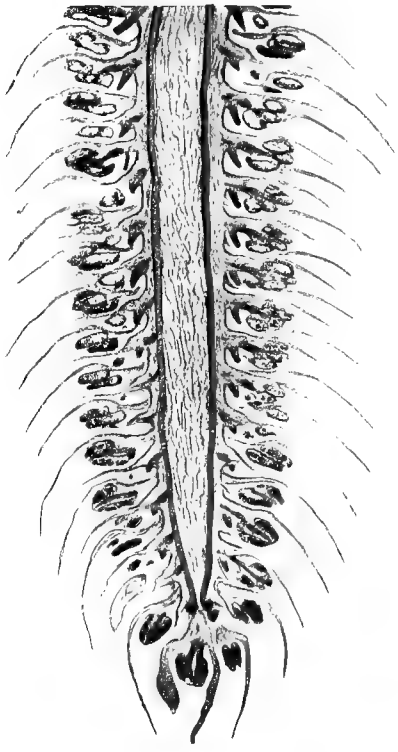

H

PLATE XVII.

FLORAL STRUCTURE.

FIG. 60. Flower, entire of Wheat. 'Triticum vulgaris' × 15 dia. Fixed with Chromo-Acetic solution. Stained with Borax Carmine. 1. Ovary. 2. Ovule with embryo-sac. 3. Enlarged base of flower. 4. Main shafts of stigma. 5. Lateral branches of stigma. 6. Anthers, having already opened and shed some of their pollen. 7. Filament of stamen. (Specimen dissected out of its sheath about ten days prior to its natural time of opening.)

FIG. 61. One shaft of a Stigma, from a flower collected and prepared as above. 1. Shaft of the stigma. 2. An isolated pollen grain, with its tube. 3. Pollen grain, its tube penetrating a stigma, its course being indicated by dotted lines.

FIG. 62. Portion of above Stigma (Fig 3 in dotted outline) × 180 dia. 3. The pollen grain. 4. Its tube; the dotted outline indicates its course. 5. Nucleus of stigmatic cell.

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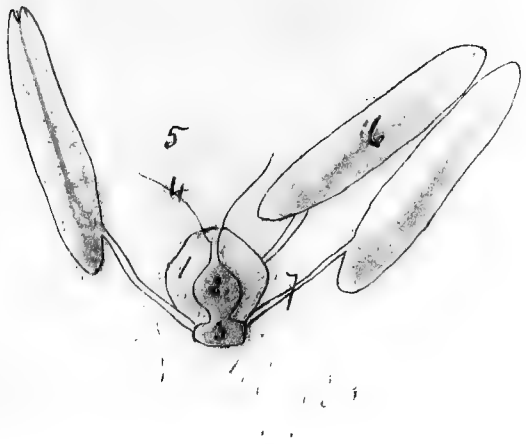

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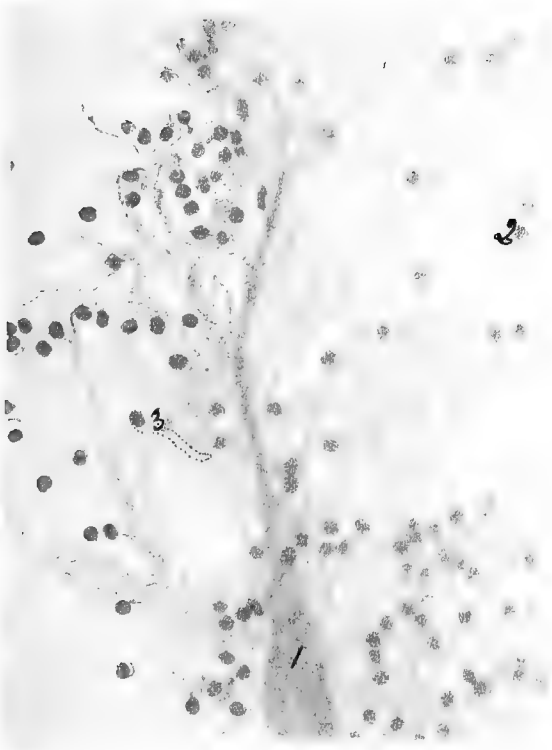

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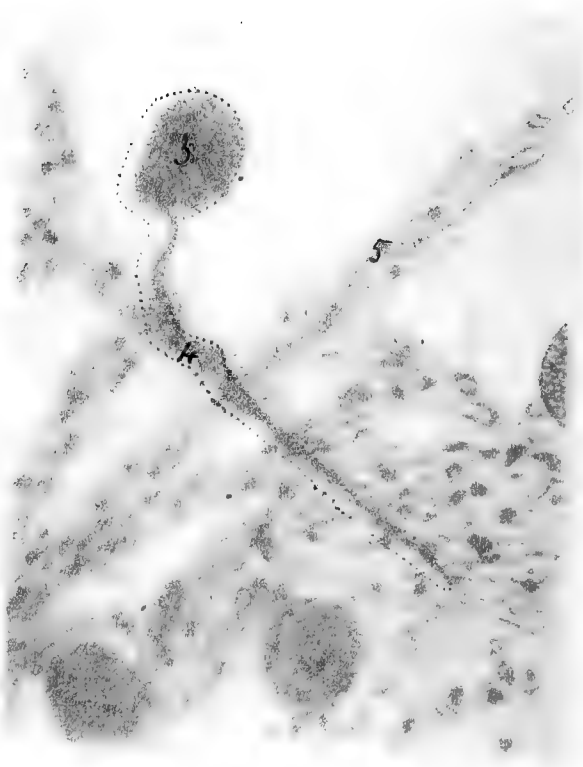
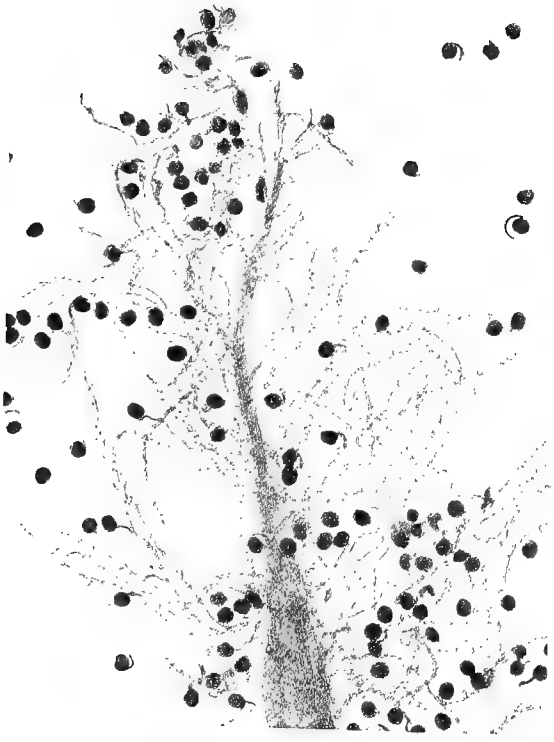




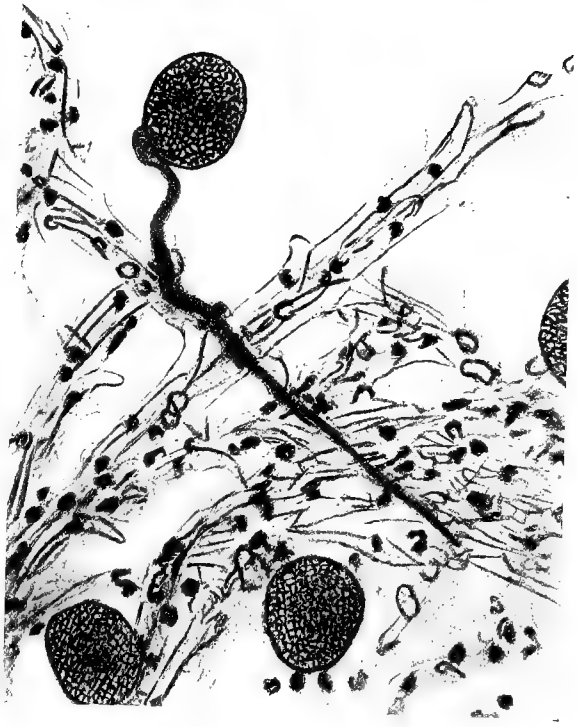


PLATE XVIII.

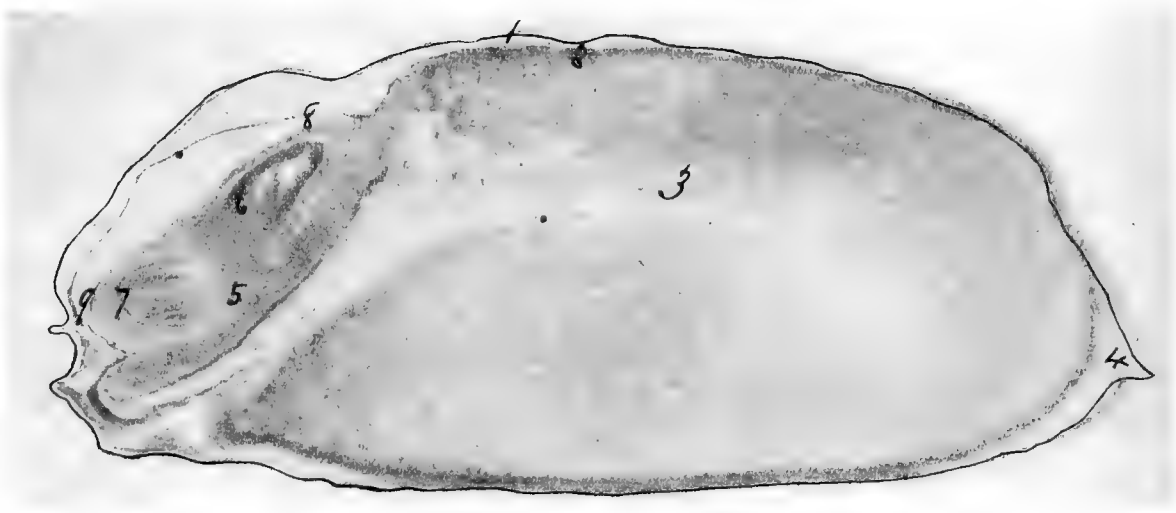
CELL STRUCTURE AND CELL CONTENTS.

FIG. 63. Longitudinal median section through a grain of Wheat. 'Triticum vulgaris' × 10 dia. (Germinated in 'milk-warm' water for sixteen hours.) Fixed with Chromo-Acetic solution. (Unstained.) 1. The pericarp. 2. Aleurone-layer. 3. Endosperm (starch containing cells). 4. Remains of stigma. 5. Scutellum. 6. The growing apex of stem. 7. Developing root. 8. Plumule sheath. 9. Root sheath.

FIG. 64. Embryo of Wheat in longitudinal median section, after germinating twenty-four hours × 40 dia. Fixed as above. Stained with Brazilin. 1. Scutellum. 2. Developing root (examine transverse section of radicals, Plate II., Fig. 5). 3. Root-cap. 4. Root sheath. 5. A developing lateral, or secondary roolet. 6. Growing apex of stem. 7. Leaves in various stages of development. 8. Plumule sheath. 9. Ligule.

FIG. 65. Transverse section through a grain of Wheat × 75 dia. Fixed as above. Stained with Brazilin. 1. Pericarp of seed. 2. Crystal. 3. Aleurone layer. 4. The endosperm cells containing starch.

63



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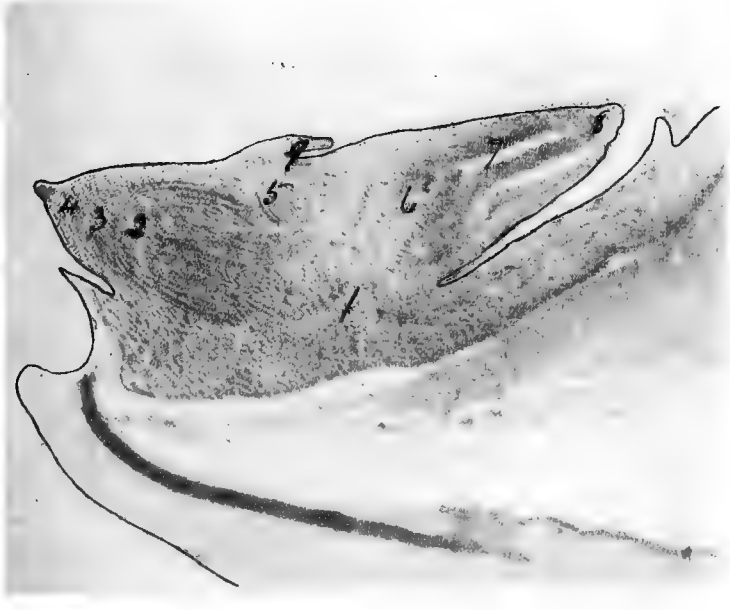

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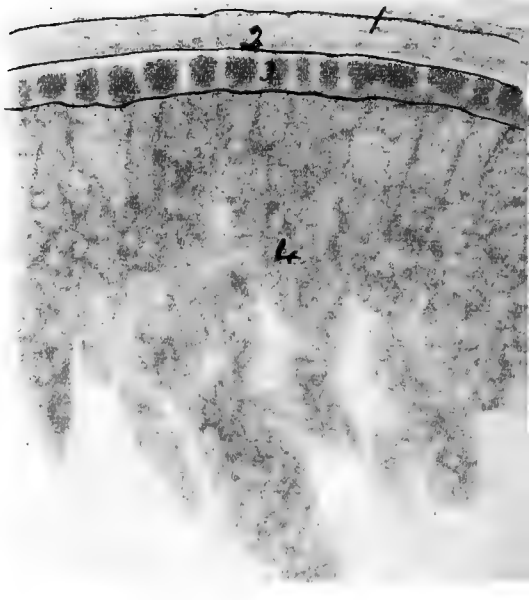


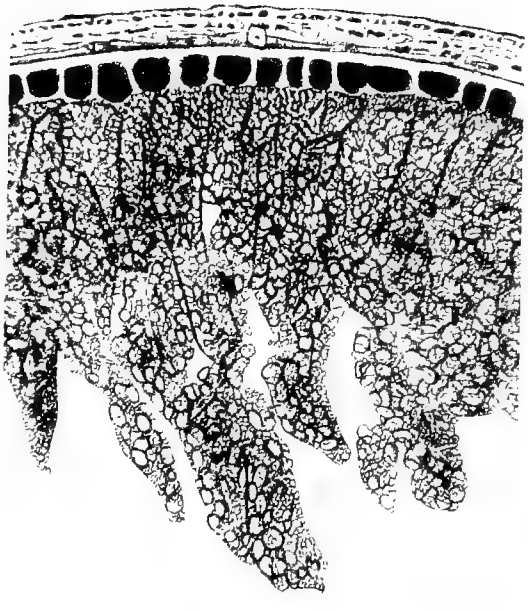


PLATE XIX.

CELL STRUCTURE AND CELL CONTENTS.

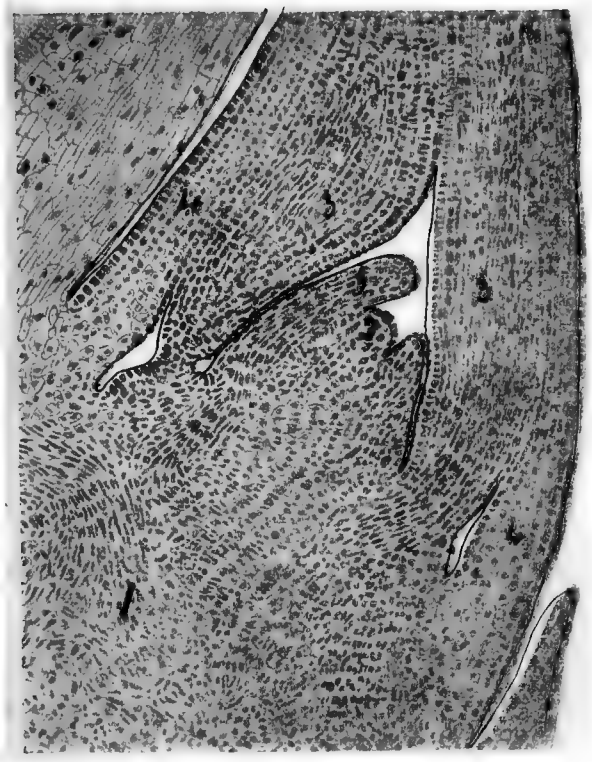
FIG. 66. Longitudinal median section through Apex of Embryo of Wheat after twenty-four hours' germination in 'milk-warm' water. Cut $^1/_{1000}$ in. × 80 dia. Fixed with Corrosive-Picro-Formaldehyde solution. Stained with Brazilin. 1. Axis of embryo.2. Growing point of stem. 3—4. Leaves in various stages of development.

FIG. 67. Longitudinal median section through radical end of Embryo of Wheat, after twenty-four hours' germination in 'milk-warm' water. Cut $^1/_{1000}$ in. × 80 dia. Fixed and stained as above. 1. Central axis of developing root. 2. Apex of root. 3. Protective root-cap. 4. Root-sheath. 5. Cells to form Epidermis (dermatogen). 6. Cells to form cortical tissue (periblem). 7. Cells to form the vascular system or root (plerom).

FIG. 68. Longitudinal section through Embryo of Wheat (before fertilisation) × 35 dia. Fixed with Chromo-Acetic solution. Stained with Brazilin. 1. Spongy tissue of ovary. 2. Ovule. 3. Young embryo.

FIG. 69. Longitudinal section through Embryo of Wheat (after fertilisation). Prepared as above. 1. Spongy tissue of ovary. 2. Embryo. 3. Disposition of cells to form seed coat.

66



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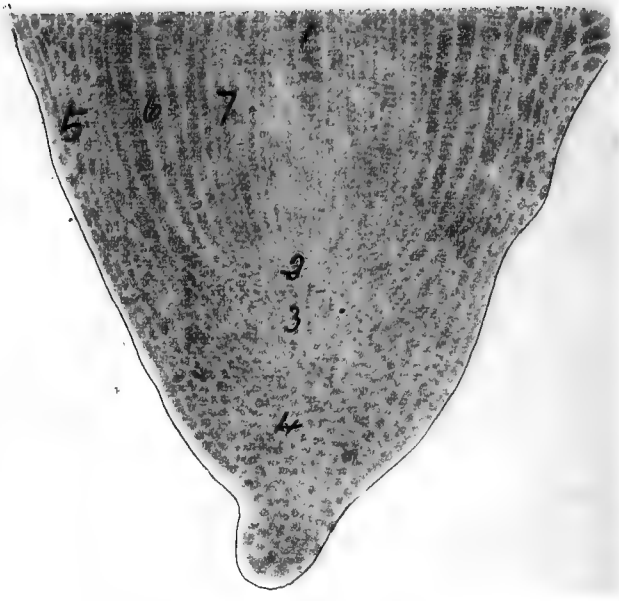

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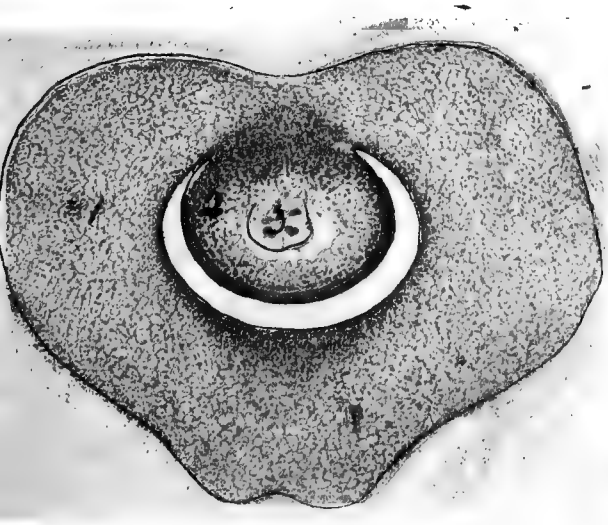

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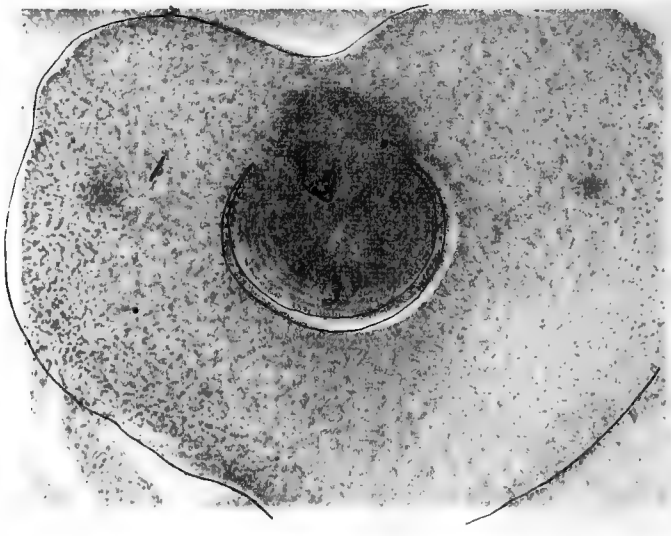
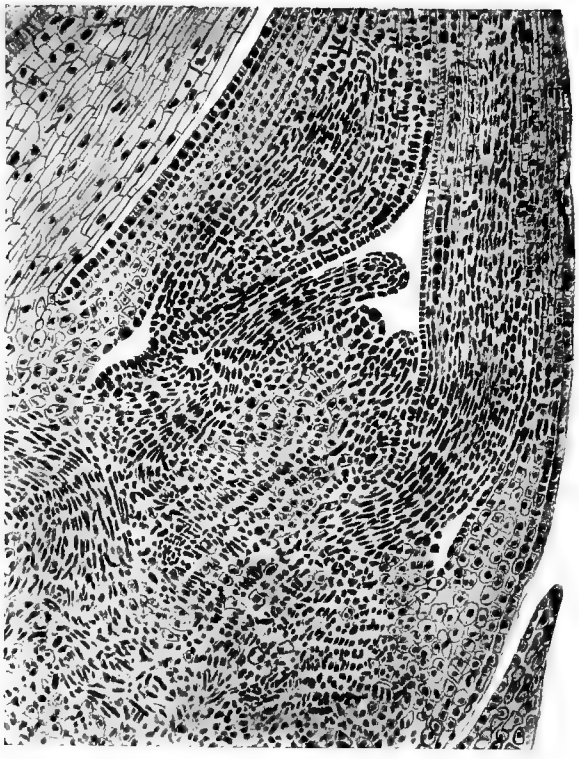
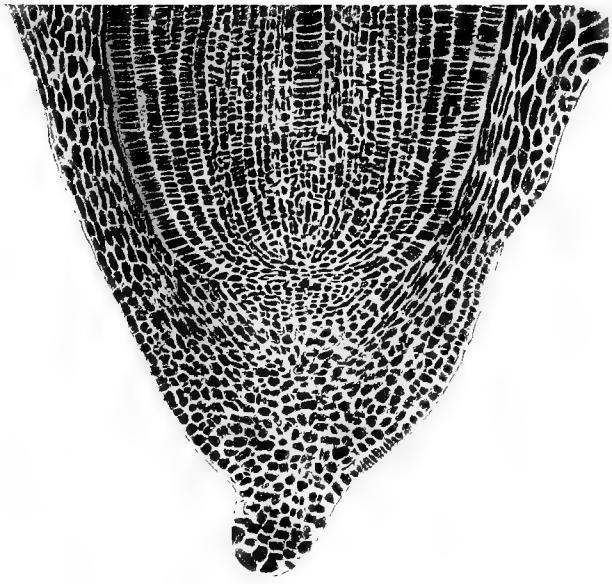
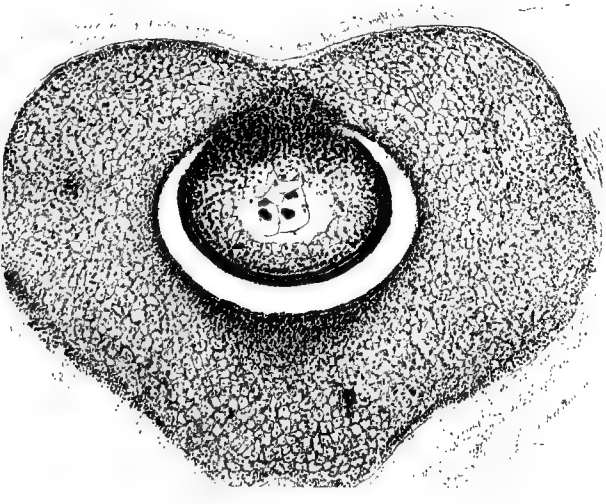








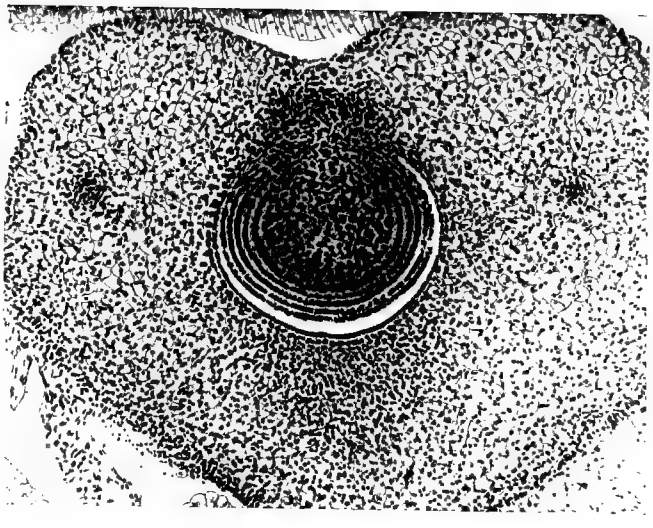


PLATE XX.

CELL STRUCTURE AND CELL CONTENTS.

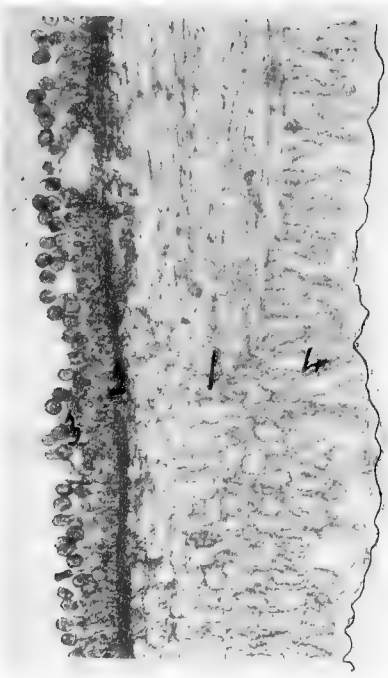
FIG. 70. Transverse section. Leaf of Thistle. 'Carduus lanceolatus,' affected with uredospores of 'Puccinia graminis' × 70 dia. Fixed with 92% Alcohol. (Unstained.) 1. disorganised tissue of leaf. 2. Mycelium of fungus. 3. Uredospores on under side of leaf. 4. Disorganised palisade layer.

FIG. 71. Transverse section. Stem of Wheat affected with 'teleuto-spores' of 'Puccinia graminis' × 70 dia. Fixed with 92% Alcohol. (Unstained.) 1. Disorganised tissue of stem. 2. Mycelium of fungus. 3. Inner layer of hollow stem. 4. Vascular bundle in normal condition. 5. Teleuto-spores.

FIG. 72. Transverse section. Leaf of Berbery. 'Berberis vulgaris,' affected with æcidiaspores of 'Puccinia graminis' × 36 dia. Fixed with 92% Alcohol. (Unstained.) 1. Ground tissue of leaf. 2. Palisade layer. 3. Mycelium of fungus. 4. Fully-developed æcidium. 5. Spermogone of unknown function.

FIG. 73. Vertical section through entire plant of 'Peziza convexula' × 60 dia. Fixed with 92% Alcohol. Stained with Aniline-Picrate and Gossypimine. 1. Thallus. 2. Sterile tissue. 3. Ascospores in various stages of development. 4. Para-physes. 5. Upper convex surface of plant.

70



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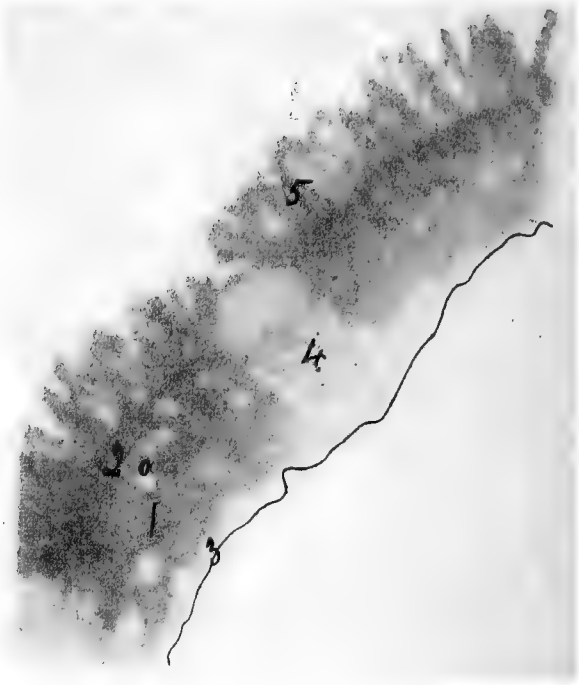

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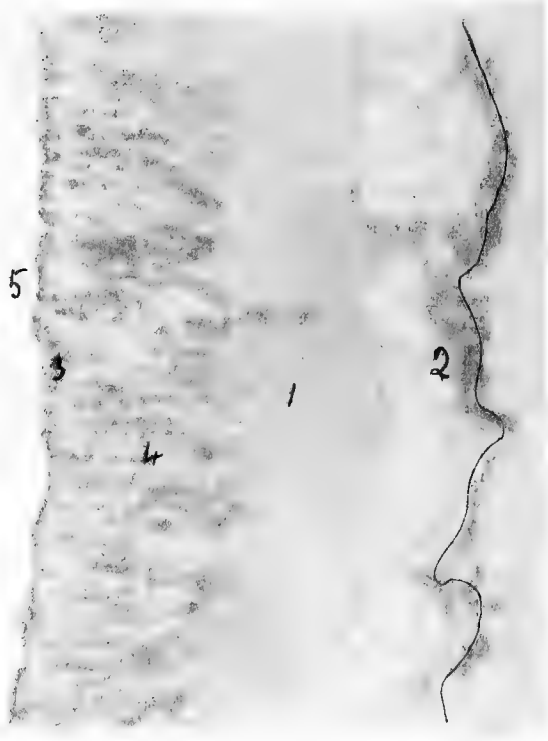
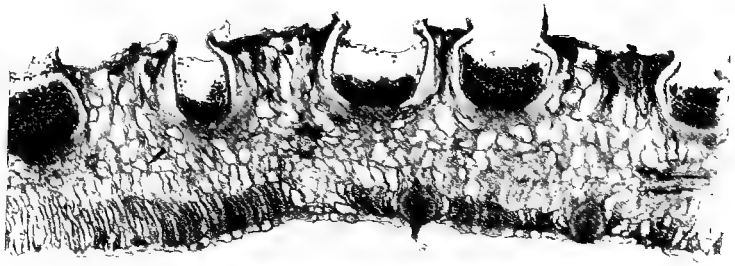
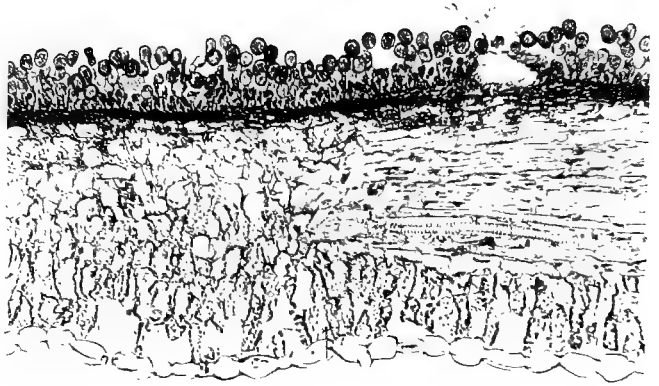
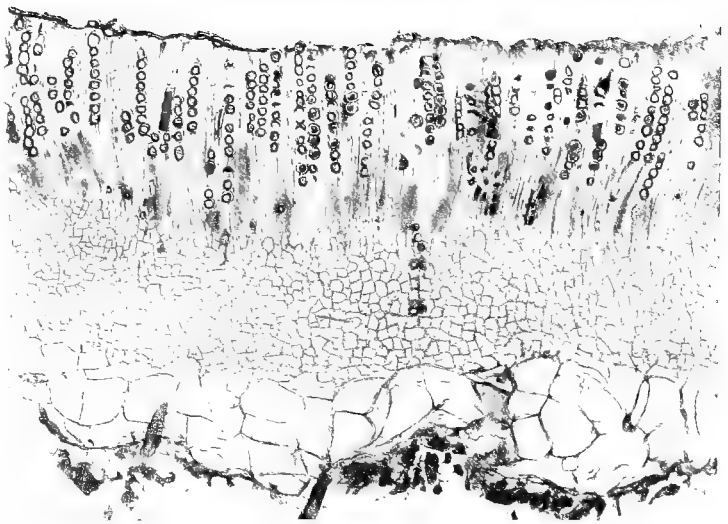








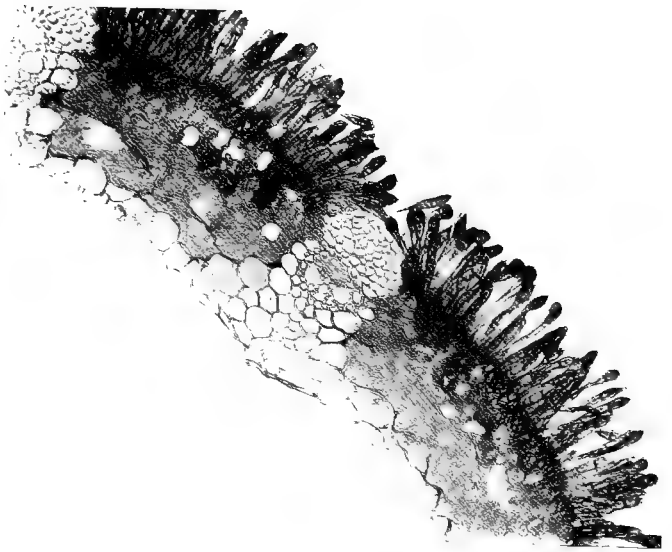


PLATE XXI.

CELL STRUCTURE AND CELL CONTENTS.

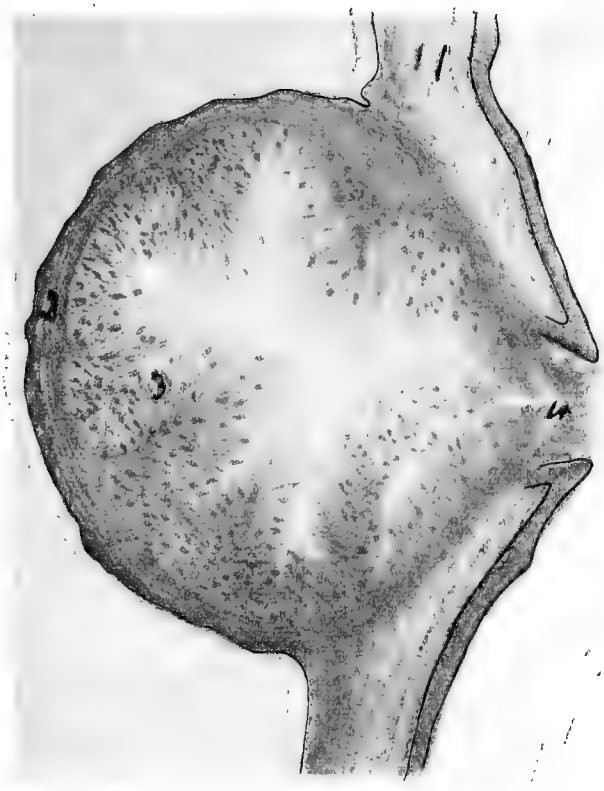
FIG. 74. Transverse section. Male conceptacle of 'Fucus vesiculosus ×60 dia. Fixed with Chromo-Acetic solution. Stained with Aniline-picrate and Gossypimine. 1. Limiting tissue of thallus. 2. Lining of conceptacle. 3. Protective hairs, among which are distributed the antheridia, stained red. 4. Mouth of conceptacle.

FIG. 75. Transverse section of female conceptacle of 'Fucus, sp.' ×60 dia. Fixed and stained as above. 1. Limiting tissue of thallus. 2. Protective hairs. 3. Oogonia in various stages of development. 4. Opening, or mouth of conceptacle.

FIG. 76. Entire thallus of Fern under surface. 'Gymnogramma sp' ×10 dia. Fixed with Chromo-Acetic solution. Stained with Borax Carmine. 1. Thallus. 2. Antheridia. 3. Archegonia. 4. Rhizoids (root hairs).

FIG. 77. Vertical section. Thallus of Fern, passing through the Archegonia × 160 dia. Fixed with Chromo-Acetic solution. Stained with Brazilin. 1. Upper surface of thallus. 2. The canal-cells of neck of Archegonia. 3. Ovum. (Section prepared by the 'cold' paraffin method and cut $^1/_{5000}$ in.)

74



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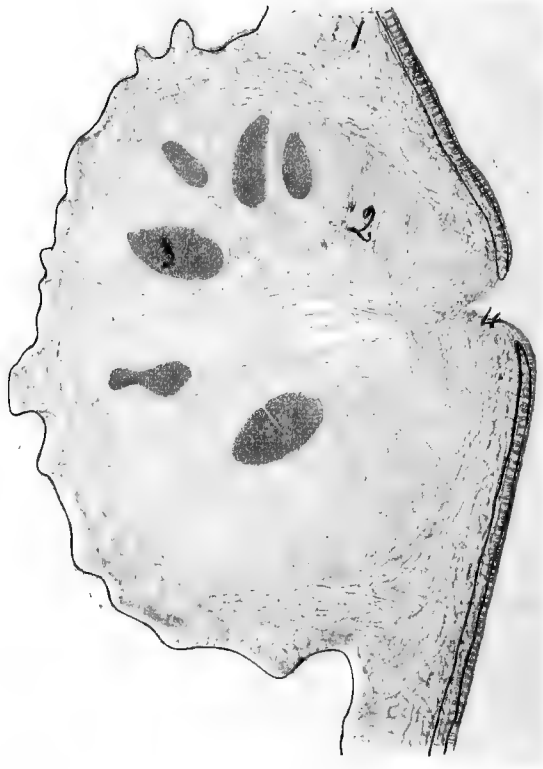

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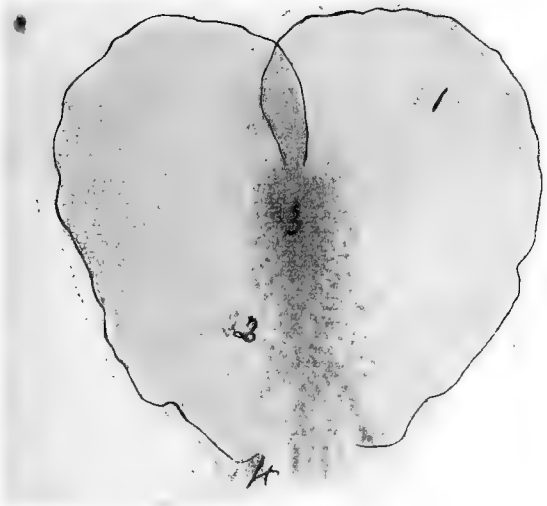

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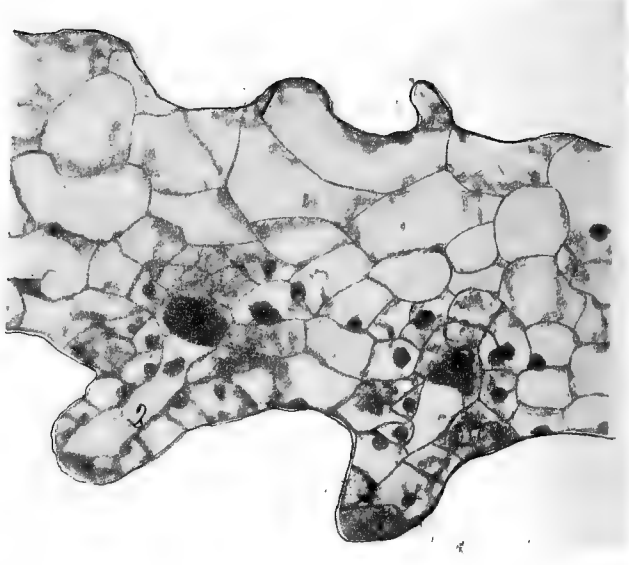
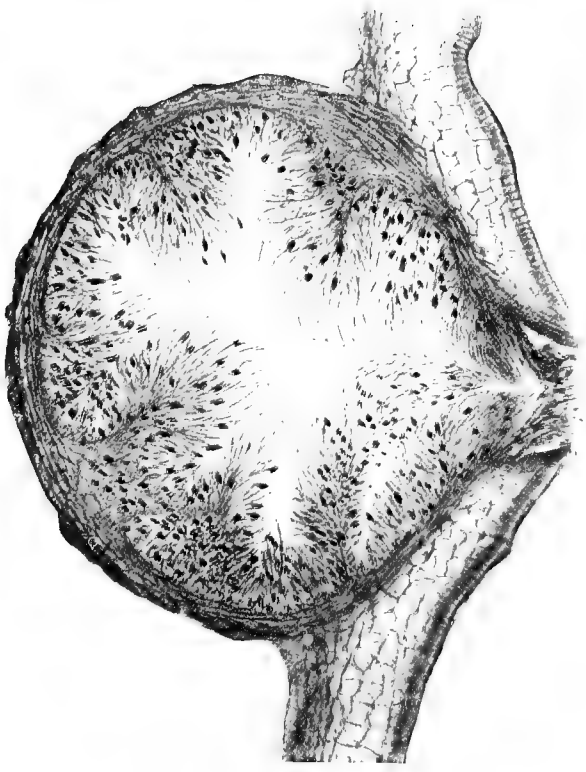
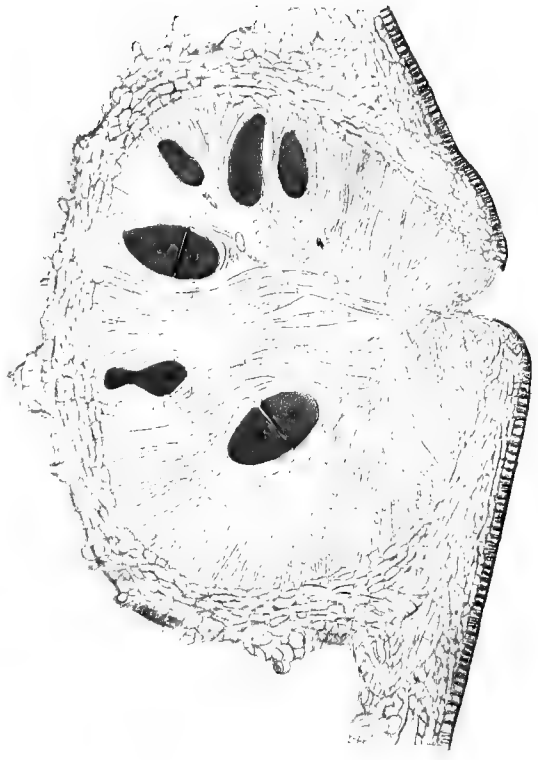
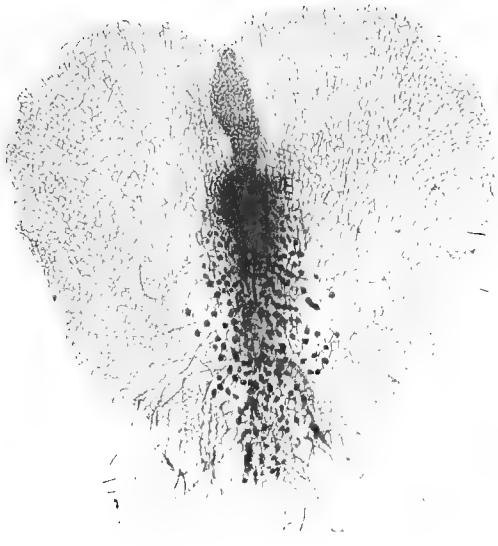








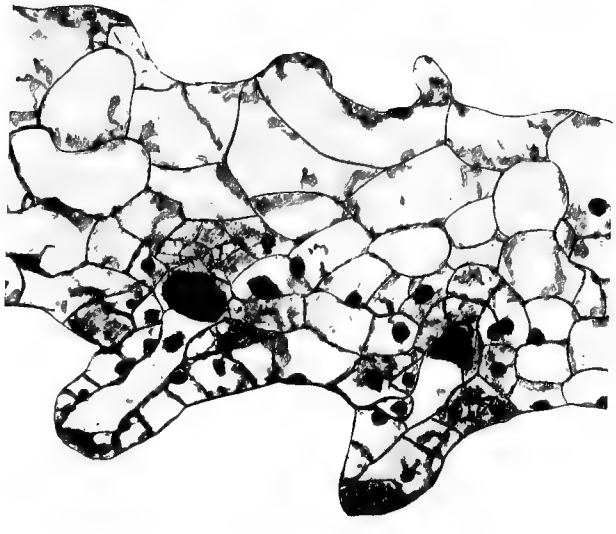


PLATE XXII.

CELL STRUCTURE AND CELL CONTENTS.

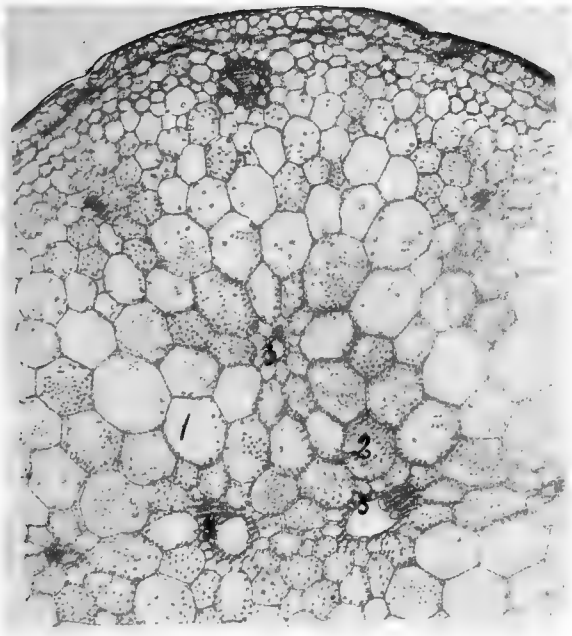
FIG. 78. Transverse section. Stem of Tradescantia. 'Tradescantia discolor' × 35 dia. Fixed with Chromo-Acetic solution. Stained with Hœmatoxylin and Gossypimine. 1. Empty cells; 2. Cells containing starch. 3. Vascular bundles.

FIG. 79. Transverse section. Stem of Pellionia. 'Pellionia Daveauna' × 65 dia. Fixed with Chromo-Acetic solution. Stained with Hæmatoxylin and Gossypimine. Starch containing cells in primary ground tissue.

FIG. 80. Longitudinal median section. Apex of Root. 'Aspidium filix-mas' × 180 dia. Fixed with Chromo-Acetic solution. Stained with Brazilin. (Cut $^1/_{6000}$ by the 'cold' paraffin method.) 1. Apical cell. 2. Zone of primary meristem tissue. 3. Protective root-cap tissue.

FIG. 81. Longitudinal section. Aerial Root of 'Monstera deliciosa' × 180 dia. Fixed with Chromo-Acetic solution. Stained with Brazilin. (Cut $^1/_{3000}$ in. by the 'cold' paraffin method.) 1. Bundles of raphides. 2. Nucleus of crystal-sac 3. Primary nucleated ground tissue. 4. Nuscleus in division.

78



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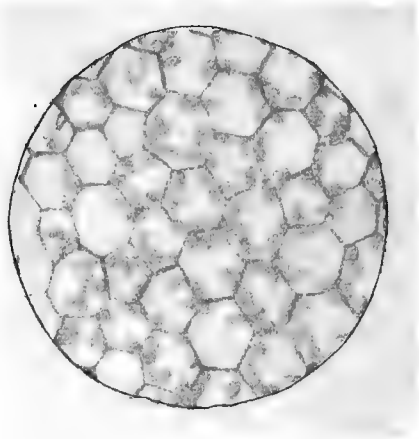

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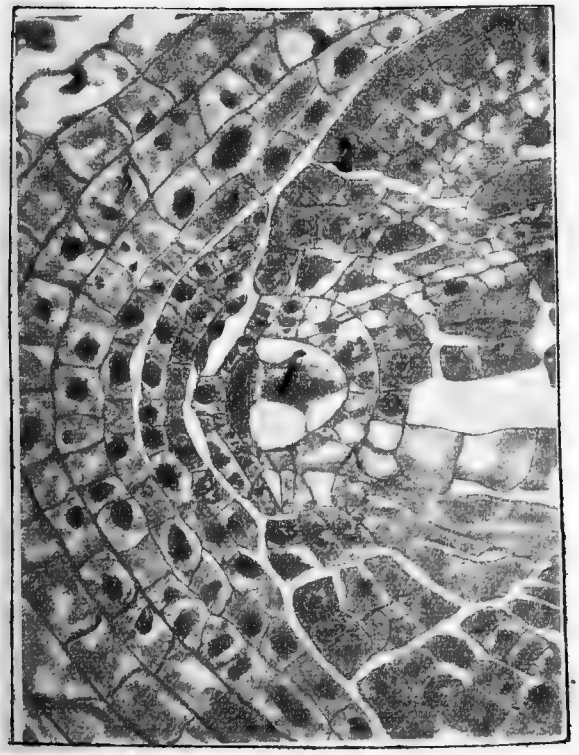

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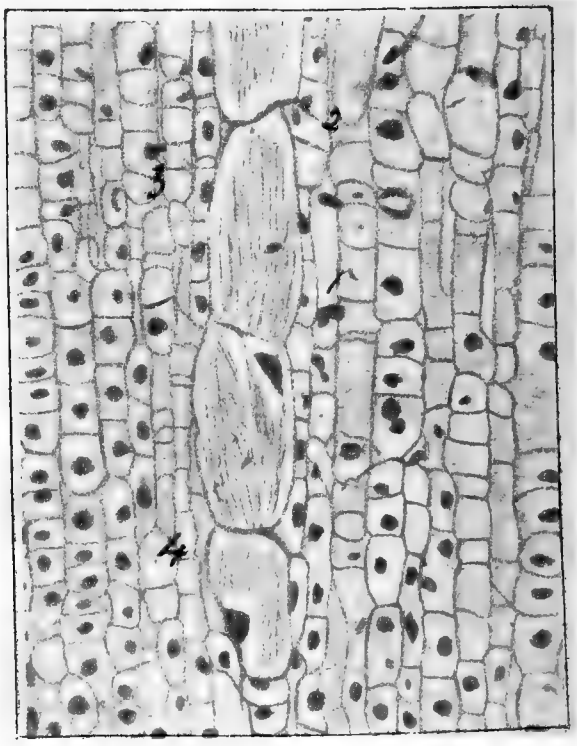
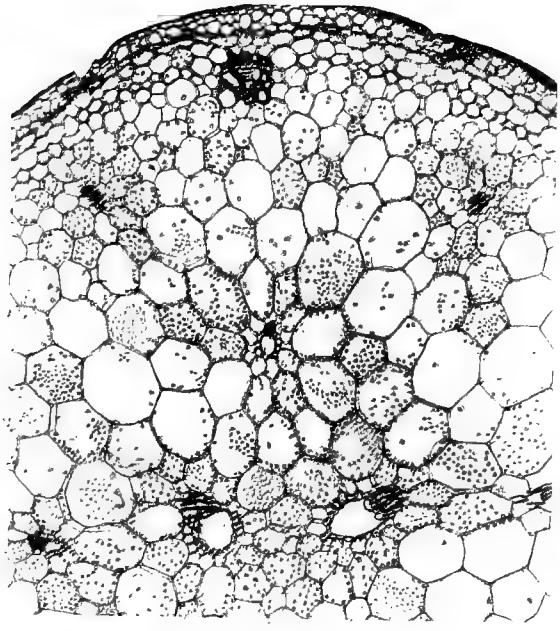
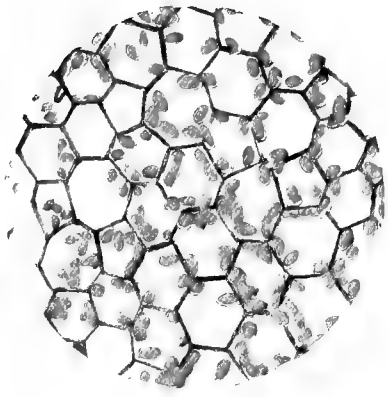
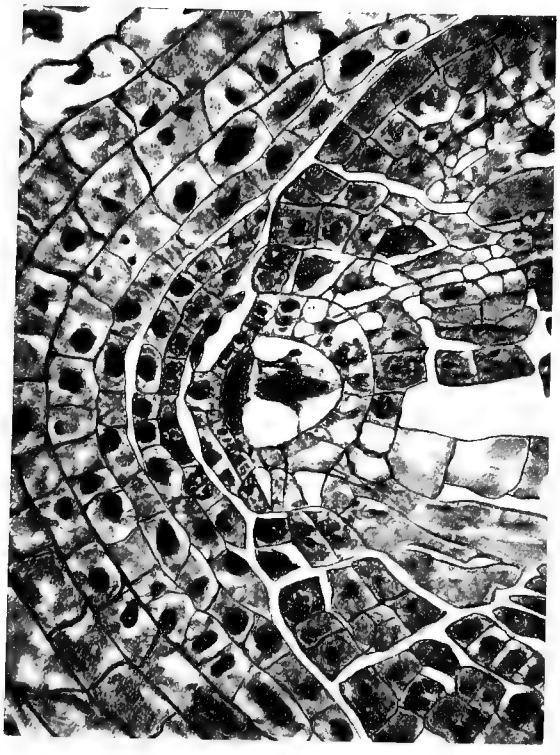








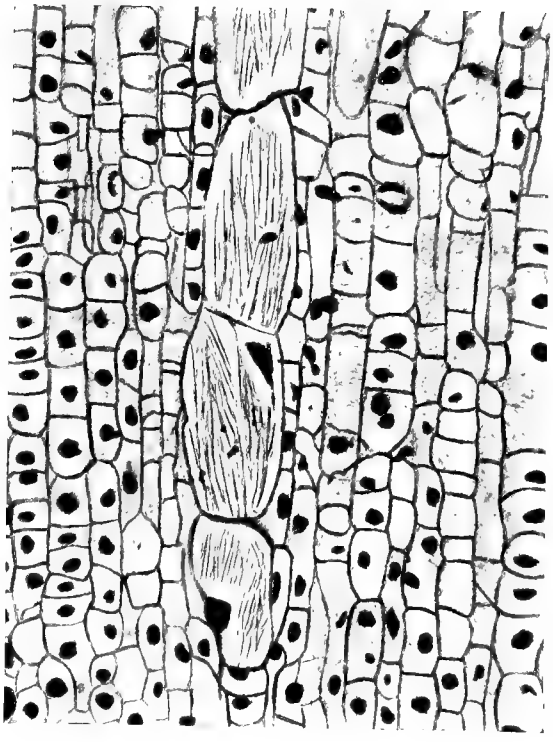


PLATE XXIII.

CELL STRUCTURE AND CELL CONTENTS.

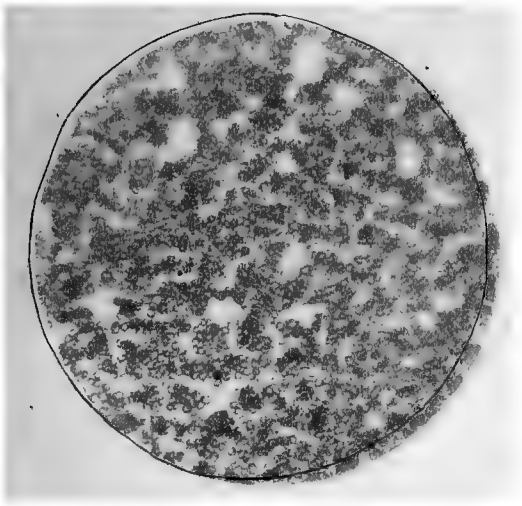
FIG. 82. Transverse section. Endosperm of 'Ricinus communis' ×45 dia. Fixed with 92% Alcohol. Unstained. Endosperm cells containing protein-crystals and aleurone grains.

FIG. 83. Longitudinal section. Root of 'Scorzonera hispanica' ×60 dia. Stained with Hœmatoxylin. 1. Primary ground tissue. 2. Laticiferous vessels.

FIG. 84. Longitudinal section. Stem of 'Euphorbia splendens' ×180 dia. Fixed with Absolute alcohol. Stained with Borax Carmine. 1. Ground tissue—cells containing starch. 2. Coagulated contents of laticiferous cell. 3. Elongated (dumb-bell) starch grains.

FIG. 85. Karyokinesis in longitudinal section of Onion. 'Allium cepa,' ×200 dia. Fixed with Chromo-Acetic solution. Prepared by the cold paraffin method. Cut $^1/_{6000}$. Stained Brazilin.

82



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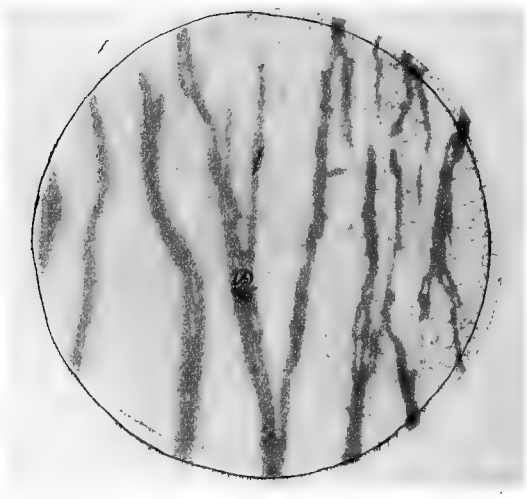

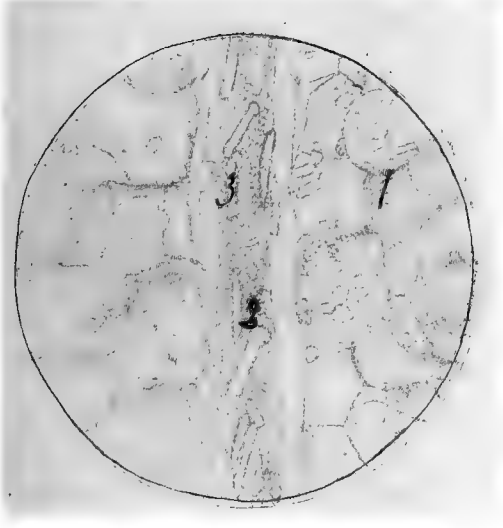

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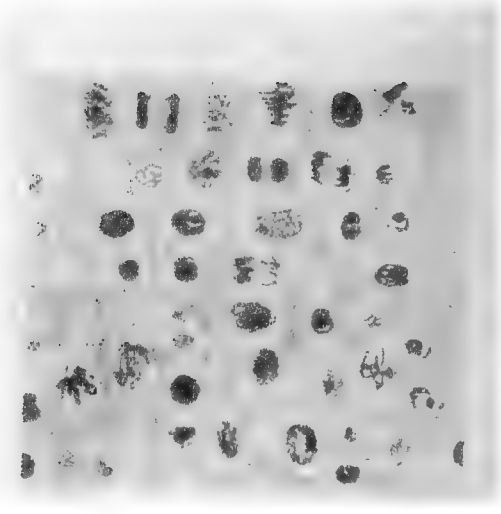

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